SAGE

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SAGE
The Genus *Salvia*

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PREFACE TO THE SERIES

There is increasing interest in industry, academia and the health sciences in medicinal and aromatic plants. In passing from plant production to the eventual product used by the public, many sciences are involved. This series brings together informa tion which is currently scattered through an ever increasing number of journals. Each volume gives an in-depth look at one plant genus, about which an area specialist has assembled information ranging from the production of the plant to market trends and quality control.

Many industries are involved such as forestry, agriculture, chemical, food, flavour, beverage, pharmaceutical, cosmetic and fragrance. The plant raw materials are roots, rhizomes, bulbs, leaves, stems, barks, wood, flowers, fruits and seeds. These yield gums, resins, essential (volatile) oils, fixed oils, waxes, juices, extracts and spices for medicinal and aromatic purposes. All these commodities are traded worldwide. A dealer's market report for an item may say "Drought in the country of origin has forced up prices".

Natural products do not mean safe products and account of this has to be taken by the above industries, which are subject to regulation. For example, a number of plants which are approved for use in medicine must not be used in cosmetic products.

The assessment of safe to use starts with the harvested plant material which has to comply with an official monograph. This may require absence of, or prescribed limits of, radioactive material, heavy metals, aflatoxins, pesticide residue, as well as the required level of active principle. This analytical control is costly and tends to exclude small batches of plant material. Large scale contracted mechanised cultivation with designated seed or plantlets is now preferable.

Today, plant selection is not only for the yield of active principle, but for the plant's ability to overcome disease, climatic stress and the hazards caused by mankind. Such methods as *in vitro* fertilisation, meristem cultures and somatic embryogenesis are used. The transfer of sections of DNA is giving rise to controversy in the case of some end-uses of the plant material.

Some suppliers of plant raw material are now able to certify that they are supplying organically-farmed medicinal plants, herbs and spices. The Economic Union directive (CVO/EU No 2092/91) details the specifications for the obligatory quality controls to be carried out at all stages of production and processing of organic products.

Fascinating plant folklore and ethnopharmacology leads to medicinal potential. Examples are the muscle relaxants based on the arrow poison, curare, from species of *Chondrodendron*, and the antimalarials derived from species of *Cinchona* and *Artemisia*. The methods of detection of pharmacological activity have become increasingly reliable and specific, frequently involving enzymes in bioassays and

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avoiding the use of laboratory animals. By using bioassay linked fractionation of crude plant juices or extracts, compounds can be specifically targeted which, for example, inhibit blood platelet aggregation, or have antitumour, or antiviral, or any other required activity. With the assistance of robotic devices, all the members of a genus may be readily screened. However, the plant material must be **fully** authenticated by a specialist.

The medicinal traditions of ancient civilisations such as those of China and India have a large armamentarium of plants in their pharmacopoeias which are used throughout South East Asia. A similar situation exists in Africa and South America. Thus, a very high percentage of the world's population relies on medicinal and aromatic plants for their medicine. Western medicine is also responding. Already in Germany all medical practitioners have to pass an examination in phytotherapy before being allowed to practise. It is noticeable that throughout Europe and the USA, medical, pharmacy and health related schools are increasingly offering training in phytotherapy.

Multinational pharmaceutical companies have become less enamoured of the single compound magic bullet cure. The high costs of such ventures and the endless competition from too many compounds from rival companies often discourage the attempt. Independent phytomedicine companies have been very strong in Germany. However, by the end of 1995, eleven (almost all) had been acquired by the multi national pharmaceutical firms, acknowledging the lay public's growing demand for phytomedicines in the Western World.

The business of dietary supplements in the Western World has expanded from the Health Store to the pharmacy. Alternative medicine includes plant based products. Appropriate measures to ensure the quality, safety and efficacy of these either already exist or are being answered by greater legislative control by such bodies as the Food and Drug Administration of the USA and the recently created European Agency for the Evaluation of Medicinal Products, based in London.

In the USA, the Dietary Supplement and Health Education Act of 1994 recognised the class of phytotherapeutic agents derived from medicinal and aromatic plants. Furthermore, under public pressure, the US Congress set up an Office of Alternative Medicine and this office in 1994 assisted the filing of several Investigational New Drug (IND) applications, required for clinical trials of some Chinese herbal prepa rations. The significance of these applications was that each Chinese preparation involved several plants and yet was handled as a single IND. A demonstration of the contribution to efficacy, of each ingredient of each plant, was not required. This was a major step forward towards more sensible regulations in regard to phytomedicines.

My thanks are due to the staff of Harwood Academic Publishers who have made this series possible and especially to the volume editors and their chapter contributors for the authoritative information.

Roland Hardman

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PREFACE

Salvia is a fascinating plant genus. One of the widest-spread members of the Labiatae family, it features prominently in the pharmacopoeias of many countries throughout the world. From the Far East, through Europe and across to the New World several of the almost 1000 *Salvia* species have been used in many ways, e.g. essential oils used in perfumery, the flowers used as rouge, the leaves used for varicose veins, the seed oil as an emollient, the roots as a tranquiliser. The range of traditional applications of the herb in domestic medicine seems to be endless: it has been used as a medication against perspiration and fever; as a carminative; a spasmolytic; an antiseptic/bactericidal; an astringent; as a gargle or mouthwash against the inflammation of the mouth, tongue and throat; a wound-healing agent; in skin and hair care; and against rheumatism and sexual debility in treating mental and nervous conditions as well as an insecticidal.

This book begins with the presentation of the (approximately 400) most known *Salvia* species (Chapter One, A.C.Dweck), their pharmacopoeial status, their history and distribution, traditional uses as a food source and in domestic medicine, as well as general information on the chemical composition of prominent *Salvia* species, such as *S. officinalis, S. bowleyana, S. coccinea, S. columbariae, S. digitaloides, S. divinorum, S. hispanica, S. horminum, S. lavandulaefolia, S. miltiorrbiza, S. plebeia, S. pomifera, S. repens, S. rugosa, S. runcinata, S. sisymbrifolia, S. sclarea, S. erotina, S. verbenaca* and *S. yunnanensis.* Analytical dosing instructions are given for each area of application.

The botany and the distribution—both global and regional—of the genus is presented along with taxonomical, chemotaxonomical, genetical and phylogenical aspects. In Chapter Two (R.Karousou *et al.*) detailed information is provided on sage species growing in Greece and the *ad hoc* main centre of origin and native distribution, the Mediterranean region. Emphasis is given on the three main species endemic in the region, namely *S. officinalis* ('Dalmatian or Garden Sage'), *S. fruticosa* ('Greek Sage') and *S. pomifera* ('Cretan Sage'), providing detailed information on their geographic distribution, morphology and essential oil composition. These species are remarkably variable and there is a vivid presentation of the climatically (temperature- and precipitation-) related high variation of the leaf mor phology and the qualitative and quantitative essential oil content (due, for example, to xerophytic adaptation). On the opposite side of the globe, Southern Africa, where traditional medicine plays a very important role in health care, is home to 30 species of the genus *Salvia*. In Chapter Three (A.K.Jäger and J.van Staden) we learn about the botany, the distribution in different climatic regions, the traditional usages and the chemistry of representative *Salvia* species, like *S. africana, S. chamelaeagmea* and *S. stenophylla,* the latter species being one of the few known sources of epi-a-bisabolol, a potent anti-inflammatory agent.

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An unusually large number of useful secondary metabolites, belonging to various chemical groups, have been isolated from *Salvia* species. These include various phenolic acids (such as caffeic, chlorogenic, ellagic, ferulic and gallic acid), tannins and volatile substances. Chapters 4–6 provide detailed information on the extraction, isolation and characterization of those components to which the biological properties of sage can be attributed: terpenoid compounds, essential oils in general and phenolic derivatives like salvianolic acids (including rosmarinic acid and lithospermic acid). In Chapter Four (A.Ulubelen), the presence of terpenoids (except of monoterpenoids) in *Salvia* species is thoroughly discussed. The chemical structure and botanical distribution and, in certain cases, structure-related bioactive properties of a total of 111 terpenoids is discussed, including various groups of diterpenoids (abietane, clerodane, pimarane and labdane-type), triterpenoids, sesquiterpenoids and sesterterpenoids. Cumulative data on the seasonal and intraspecific variation of essential oils are also included (Chapter Five, A.L. Giannouli and S.E.Kintzios), indicating that it may be possible to manipulate essential oil content in such a way that heavy investment of time and resources in selection and breeding can be avoided. In Chapter Six (Lian-Niang Li) the chemistry of the bioactive polyphenolic acids of various *Salvia* species, in particular *S. miltior rhiza,* is presented. These substances are commonly known as rosmarinic acid, lithospermic acid, salvianolic acids (A-J) and related compounds. Detailed information is provided on their chemical structure, extraction and isolation methods, UV-, MS- and NMR-spectra and chemical transformation.

The optimization of tillage, harvest and dry process technologies, as well as the proper application of fertilizers and pesticides largely improved the quality of the raw material. Chapter Seven (A.J.Karamanos) constitutes an in-depth review of virtually every aspect concerning the cultivation technology of sage, such as propa gation, land preparation, irrigation, fertilizer, growth regulator and herbicide application, harvest and postharvest treatment. A special reference is made to the ecophysiology of the genus in respect of its response to abiotic and biotic stress factors (drought, heavy metals, light, temperature, allelopathy, etc.) and their effect on biomass production and product yield and quality (e.g. essential oil composition) (Chapter Nine, E.Panagiotopoulos *et al.*).

Regarding optimization of the cultivation of the species for appropriate plant material, initially only indigenous local or introduced populations were used with moderate efficacy. To provide the basis for economical production, the breeding work especially on *S. sclarea* and *S. fficinalis* became more intensive. Breeding work on the genus is currently done in some countries (mainly Eastern European ones) to obtain varieties with improved characteristics, in their agricultural behaviour as well as in their chemical composition. In Chapter Eight (J.Bernáth and É.Németh) we see how various selection goals are approached by different strategies and methods country by country, depending on the local tradition and experiences. The high morphological and chemical diversities of the species are utilised, even if the plant is growing wild locally, or the indigenous populations had been introduced from exterior habitats. In particular there is extensive reference to the utilization of morphological and chemical diversity of indigenous populations as a background

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for genetic improvement, the improvement of populations by selection, the creation of new cultivars by hybridisation, the construction of polyploid forms and mutation breeding. A continuous breeding effort, incorporating both classical and modern, unconventional methods, led to the creation of new productive varieties (such as 'Extrakta', a *S. officinalis* cultivar which is particularly rich in essential oil). Breeding new sage varieties requires substantial investment in terms of skill, labour, material resources and money and may take years. An efficient protection of the intellectual property should enhance the breeding work on the *Salvia* genus. The 1991 Act of the UPOV Convention and the 1994 TRIP'S Agreement provide for the possibility of protecting all genera and species in many countries. Hence, this gives a new opportunity to breeders of ornamental, medicinal and aromatic plants and in particular to *Salvia* breeders. The current situation of protection with a detailed explanation of the UPOV plant protection system and its main dispositions are given in Chapter Ten (B.Le Buanec).

The biological effects of plant extracts and/or essential oils and other important compounds of various species of the genus *Salvia* have been acknowledged over the centuries. Besides *S. officinalis,* which is additionally referred to as having antibiotic properties, other species also contain compounds with important pharmaco logical activities. In Chapter Eleven (D.Baricevic and T.Bartol), the bioactive/pharmacological properties of the genus are quite extensively reviewed, including antimicrobial, antiviral, cardiovascular, renal, antioxidative, anti-inflammatory, tumorigenesis-preventing, antimutagenic, peptic-antiulcer, antispasmodic, hypoglycemic and hepatoprotective activities, as well as documented toxic effects. Extension of the use of sage as a food additive or a herbal medicine has been prevented mostly due to the toxic effects of the ketone terpenoids in the volatile oil, namely camphor and thujone. A separate section of this chapter is devoted to the description of the pest-toxic and repellent activities of the genus.

Because of the unknown effect of synthetic antioxidants like butylated hydroxyanethole [BHA] and butylated hydroxytoluene [BHT] on human cancer risk the interest in preparing antioxidants from natural sources with minimal processing has considerably increased in recent years. Sage and related species are an important source of antioxidants used in the food industry and have wider implications for the dietary intake of natural antioxidants. *Salvia* is one of the favourite candidate species as a source of natural antioxidants in health care products. In Chapter Twelve (S.G.Deans and E.J.M.Simpson) the chemical structure, isolation and activity of the major antioxidant compounds of sage, mainly rosmarinic acid, carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol and methyl carnosate, are presented. Aspects of essential oil variability and herbal material purity due to geographic location and drying temperature are investigated, along with a short description of assays for the determination of antioxidant activity. There is an analytical reference to studies investigating the relationship between leaf senescence and the oxidative defense system of sage, as well as the activity of the antioxidants in improving the responsiveness of the human immune system and reducing the free radical damage.

Dan-Shen extracts, derived from the roots of *S. miltiorrhiza,* have traditionally been used to treat haematological abnormalities and cardiovascular diseases in

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China. Along with tanshinone IIA sodium sulfonate, magnesium lithospermate B (a tetramer of caffeic acid) is an important constituent with antihypertensive properties. In Chapter Thirteen (T.Yokozawa) the structure and activity of this and related compounds is evaluated in detail, presenting its effects on blood flow and renal function as a result of the interaction with the secretion of prostaglandin E_2 and kallikrein activation.

Sage is also renowned for its effects on the central nervous system. Various anxiolytic and sedative, memory-enhancing, antidepressive and hallucinogenic activities have been ascribed to the genus (Chapter Fourteen, N.Perry *et al.*), thus making it a promising ingredient in the future treatment of CNS-related ailments.

Biotechnological techniques have been recently reported to significantly facilitate plant propagation and production of some important bioactive compounds of the genus *Salvia*. A special chapter of the book (Chapter Fifteen, O.Makri) is devoted to reviewing the biotechnology research that has been done in various other species of the Labiatae family, such as *Mentha, Origanum, Thymus, Lavantula, Ocimum, Hyssopus* and *Coleus,* whose antioxidant activity is valuable for the food, cosmetic and pharmaceutical industries.

Rosmarinic acid can be produced by cell suspension cultures of sage. The growth and production of rosmarinic acid by sage cells is modified by the type of culture medium used (Chapter Sixteen, I.Hippolyte). Rosmarinic acid production is increased 10-fold to attain 6.4 gL^{-1} (or 36% of the dry weight) under optimal conditions. Investigation of cell growth kinetics showed that a change in the medium caused shifts in peaks of growth and rosmarinic acid production, and modifications in cellular metabolism. By changing the composition of the culture medium it is possible to manipulate rosmarinic acid production to coincide with cell growth or to begin only when growth had stopped.

There is an increasing interest in the development of efficient protocols for the tissue culture and micropropagation of certain *Salvia* species, in order to establish a relatively fast system for producing disease-free and true-to-type clonal (and therefore uniform) plants from outstanding genotypes. Progress in somatic embryogenesis and recent research on the technology of synthetic seeds, along with other advanced aspects of tissue culture (e.g. protoplast culture and fusion, creation of autotetraploid lines) could offer a significant involvement of biotechnology in the propagation and breeding of the genus *Salvia.*

Chapter Seventeen (S.E.Kintzios) offers a concise presentation of the various methods developed for the induction of callus, organogenesis and somatic embryogenesis as well as plant regeneration for micropropagation and breeding purposes of some *Salvia* species. Furthermore, the accumulation of secondary metabolites (in particular rosmarinic and lithospermic acid) in *in vitro* differentiated tissues is reviewed.

Biotechnology opens new perspectives for an automated, scaled-up and cost-efficient production of useful compounds from *Salvia* spp. Cell suspensions, immob ilized cell and hairy root cultures have been established from *S. officinalis, S. miltiorrhiza, S. fruticosa* and *S. sclarea* and used for the production of various secondary metabolites, such as rosmarinic acid, cryptotanshinone, ferruginol

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(achieving a yield of 29, 101 and 254 g/1, respectively) and (commercially) sclareol. Scale-up and immobilization techniques for *Salvia* liquid cell culture are the focus of Chapter Eighteen (E.Panagiotopoulos *et al.*), where updated information on the *in vitro* secondary metabolism of various important compounds can also be found.

It is hard to describe sage as an industrial crop, since its worldwide production is less than 25 000 kg per year. There is, however, a steady upward trend in the export of essential oil from various sage species, for use as products in the aromatherapy and natural cosmetics market. Commercial sage species include *S. officinalis, S. fruticosa, S. lavandulaefolia, S. verbenaca, S. sclarea* and *S. tomentosa*. In Chapter Nineteen (K.H.C.Baser) the actual situation of sage oil production and export (mainly in Mediterranean countries) is presented. In Turkey alone, approximately 500 kg of leaf oil from *S. triloba (S. fruticosa)* is annually produced and 600 tonnes of sage leaves worth more than 1.5 million US$ is exported. There is, in addition, an increasing demand for herbal tea from organically grown sage.

In the age of information technology a researcher is facing an accelerating growth of all kinds of scientific and technical data. The average yearly growth of *Salvia*-related publications is 2.2%. This rate is slight but persistent and shows a con tinuing presence of interest in the field. In an exhaustive analysis (Chapter Twenty, T.Bartol and D.Baricevic) most major bibliographic life-sciences databases are identified and assessed as pertinent sources for information on the genus *Salvia*. Using the example of *Salvia,* the degree of overlap across databases is observed and the annual trend of publishing for this genus and major journals where *Salvia*-related articles have been published is identified. An investigation of these databases and their differences in relation to keyword or classification representation of the topics is conducted. Finally, the most appropriate search technique in order to maximize the recall and optimize the precision is selected and presented.

Spiridon E.Kintzios

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Dedicated to Katia

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I. INTRODUCTION
1. THE FOLKLORE AND COSMETIC USE OF VARIOUS *SALVIA* SPECIES

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SALVIA SPECIES

Salvia acetabulosa
Salvia acinos
Salvia acuminata Ruiz & Pav.
Salvia acutifolia Ruiz & Pav.
Salvia adenoclada Briq.=*Salvia striata* Benth.
Salvia aegyptiaca
Salvia aegyptiaca
Salvia aethiopis L.
Salvia alata Epling
Salvia albimaculata
Salvia albo-caerulea
Salvia alborosea Epling & Jativa
Salvia amarissima
Salvia amethystina
Salvia amplexicaulis
Salvia apiana
Salvia arabica
Salvia areysiana
Salvia argentea L.
Salvia arisanensis
Salvia arizonica Arizona sage
Salvia aspera
Salvia atrocalyx Epling
Salvia aucheri
Salvia austriaca Jacq.
Salvia axillaris
Salvia ayavacensis Kunth [Syn. *Salvia ayavacensis* Epling]
Salvia aytachii
Salvia azurea Azure Sage
Salvia azurea var. *grandiflora*
Salvia ballotaeflora

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Salvia ballotiflora
Salvia bariensis
Salvia biflora Ruiz & Pav.=*Salvia tubiflora* Ruiz & Pav.
Salvia biflora var. *glabrata* Benth.=*Salvia tubiflora* Ruiz & Pav.
Salvia blancoana
Salvia blodgettii
Salvia bodinieri Vaniot
Salvia bogotensis
Salvia booleana
Salvia bowleyana Dunn
Salvia brandegei
Salvia breviflora
Salvia broussonettii
Salvia bucharica
Salvia bullulata Benth. [Syn: *Salvia bullulata* Benth.]
Salvia cadmica
Salvia caespitosa Montbret et Aucher ex Benth.
Salvia calocalicina Briq.=*Salvia paudserrata* subsp. *calocalicina* (Briq.)
Salvia calycina
Salvia camporum Epling
Salvia canariensis L.
Salvia candelabrum Spanish Sage
Salvia candica
Salvia candicans
Salvia candidissima
Salvia cardiophylla
Salvia carduacea
Salvia carnea
Salvia carnosa
Salvia cavalerici
Salvia cbapmanii
Salvia chia
Salvia chicamochae
Salvia chinensis
Salvia claytoni
Salvia clevelandii (Gray) Greene Blue Sage
Salvia clevelandii Fragrant Sage
Salvia coccinea Blood Sage, Tropical Sage
Salvia coccinea Buc'hoz ex Etlinger
Salvia coccinea var. *pseudococcinea* (Jacq.) A.Gray
Salvia columbariae Benth. California sage
Salvia columbariae Chia, Golden Chia
Salvia compressa
Salvia confertiflora
Salvia consobrina Epling

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Salvia corrugata Vahl
Salvia cruikshanksii Benth.
Salvia cryptantha Montbret et Aucher ex Benth
Salvia cupheifolia Kunth=*Salvia oppositiflora* Ruiz & Pav.
Salvia cuspidata Ruiz & Pav.
Salvia cyanicalyx Epling
Salvia cylindriflora Epling
Salvia cypria Cyprus Sage
Salvia dazlyi
Salvia deserta
Salvia desoleana
Salvia digitaloides Diels
Salvia discolor Kunth
Salvia divaricata
Salvia divinorum Epling et Jativa Mexican Mint, Pipilzintzintli, Holy Sage
Salvia dombeyi Epling [Syn: *Salvia dombeyi* R. & P.]
Salvia dominica
Salvia dorisiana
Salvia dorrii Gray Ball Sage
Salvia dorrii ssp. *argentea*
Salvia dorrii ssp. *carnosa*
Salvia dorrii ssp. *dorrii* Gray Ball Sage
Salvia dorrii ssp. *gilmanii*
Salvia dorrii var. *carnosa*
Salvia dracocephaloides
Salvia elegans Pineapple Sage
Salvia eremostachya
Salvia ermenekensis
Salvia esquirolii Lévl.
Salvia euphratica
Salvia excisa Ruiz & Pav.=*Salvia tubiflora* Ruiz & Pav.
Salvia falcata
Salvia farinacea Mealy-Cup Sage
Salvia flava
Salvia flocculosa Epling & Mathias=*Scutellaria flocculosa* Epling & Mathias
Salvia florida Benth.
Salvia fluviatilis
Salvia formosa L'Heritier [Syn: *Salvia formosa* Gloxin]
Salvia formosa L'Heritier [Syn. *Salvia formosa* Ruiz & Pav.]
Salvia forskahlei
Salvia fruticosa Miller
Salvia fruticulosa
Salvia fulgens Mexican Red Sage
Salvia funerea Death Valley Sage
Salvia garedzhi

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Salvia gilliessi or *gilliesii*
Salvia glabricaulis
Salvia glutinosa Hardy Sage
Salvia glutinosa Jupiter's Distaff
Salvia glutinosa Yellow Sage, Hardy Sage
Salvia glutinosa L.
Salvia grahamii
Salvia grandiflora Balsamic Sage, Broad-leafed Sage
Salvia grata M.Vahl=*Salvia oppositiflora* Ruiz & Pav.
Salvia gravida
Salvia greatae
Salvia greggii Autumn Sage
Salvia grisea Epling & Mathias
Salvia griseifolia Epling [Syn: *Salvia griseifolia* C.Presl ex Benth.]
Salvia guaranitica
Salvia haenkei Benth.
Salvia hapalophylla Epling
Salvia hastaefolia Epling=*Salvia rhodostephana* Epling
Salvia hayatana
Salvia heerii Regel
Salvia henryi Crimson Sage
Salvia herrerae Epling
Salvia hians
Salvia hidalgensis
Salvia hirta Kunth
Salvia hispanica L.Chia
Salvia horminum Red Topped Sage
Salvia hualiensis
Salvia hypargeia
Salvia hypoleuca
Salvia hyptoides
Salvia incurvata Ruiz & Pav.
Salvia indica
Salvia innoxia Epling & Mathias
Salvia integrifolia Ruiz & Pav.
Salvia jaimehintoniana=*Salvia azura* var. *mexicana*
Salvia japonica
Salvia jorgehintoniana
Salvia jurisicii Kosanin
Salvia keerlii
Salvia kietaoensis
Salvia korolkovii
Salvia lachnostoma Epling
Salvia lanata
Salvia lanceolata

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Salvia lanicaulis Epling & Jativa
Salvia lanigera
Salvia lavandulae
Salvia lavandulifolia Spanish Sage
Salvia lavanduloides
Salvia lemmonii Lemmon's sage
Salvia leonuroides Gloxin=*Salvia formosa* L'Heritier
Salvia leucantha Mexican Bush Sage
Salvia leucoclada Benth.=*Salvia cruikshanksii* Benth.
Salvia leucophylla Greene
Salvia limbata
Salvia lobbii Epling
Salvia longiflora R. & P.=*Salvia dombeyi* Epling
Salvia longipedicellata
Salvia longispicata
Salvia longistyla
Salvia lupulina
Salvia lycioides Canyon sage
Salvia lyrata Lyre-leaf sage, Wild Sage, Cancerweed,
Lyre Leaf Sage, Kasvaa Pohjois-Amerikassa
Salvia macbridei Epling=*Salvia revoluta* Ruiz & Pav.
Salvia macrophylla Benth. [Syn: *Salvia macrophylla* Benth.]
Salvia macrophylla var. *malacophylla* Benth.
=*Salvia macrophylla* Benth.
Salvia macrosiphon
Salvia madrensis
Salvia major
Salvia malacophylla Benth.
Salvia mathewsii Benth.=*Salvia speciosa* C.Presl ex Benth.
Salvia medusa Epling & Jativa
Salvia melaleuca
Salvia mellifera Black Sage
Salvia mellifera Greene California Black Sage
Salvia merjamie
Salvia mexicana
Salvia micrantha
Salvia microphylla
Salvia microstegia
Salvia miltiorrhiza Bunge Danchen, Danshen, Tan Shen, Tan Zhen,
Astral Sage
Salvia miltiorrhiza Bunge [Syn. *Salvia tanshen* Max.]
Salvia miltiorrhiza Bunge [Syn. *Salvia pogonocalyx* Hance]
Salvia mirzayani
Salvia misella Kunth [Syn: *Salvia misella* Kunth]
Salvia mitis Ruiz & Pav.=*Salvia punctata* Ruiz & Pav.

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Salvia mohavensis
Salvia montebrettii
Salvia moorcroftiana
Salvia moschata
Salvia mucidistachys Epling=*Salvia ayavacensis* Kunth
Salvia munzii
Salvia myuzii or mynzii
Salvia nemorosa Woodland sage
Salvia nemorosa L.
Salvia neurepia
Salvia nicolsoniana
Salvia nipponica
Salvia nodosa Ruiz & Pav.=*Salvia formosa* L'Heritier
Salvia nubigena
Salvia nutans L.
Salvia obumbrata Epling [Syn: *Salvia obumbrata* Epling]
Salvia occidentalis West Indian sage
Salvia occidentalis Sw. [Syn: *Salvia occidentalis* Ruiz & Pav.]
Salvia ochrantha Epling
Salvia officinalis L.Sage, Salvia, Broadleaf Sage, Common Sage, Dalmatian Sage, Garden Sage, Ryytisalvia
Salvia officinalis var. *rubia* Broad-Leafed Sage, Dalmatian Sage, Garden Sage, Red Sage, Sawge, True Sage
Salvia oppositiflora Ruiz & Pav. [Syn. *Salvia oppositiflora* Kunth]
Salvia oppositiflora Ruiz & Pav. [Syn. *Salvia oppositiflora* M.Vahl]
Salvia oppositiflora Ruiz & Pav. [Syn: *Salvia oppositiflora* Hooker]
Salvia oxyodon
Salvia pachypbylla Rose Sage
Salvia palaefolia
Salvia palaestina
Salvia paposana Philippi [Syn. *Salvia paposana* Benth.]
Salvia paryskii
Salvia patens Gentian Sage
Salvia patens Cav.
Salvia pauciserrata Benth.
Salvia pauciserrata subsp. *calocalicina* (Briq.) J.R.I.Wood & Harley [Syn: *Salvia pauciserrata calocalicina* Briq.]
Salvia pauciserrata var. *pauciserrata*
Salvia pavonii Benth.
Salvia penduliflora Epling
Salvia perlucida Epling
Salvia persipolitana
Salvia petiolaris Kunth=*Salvia scutellarioides* Kunth
Salvia phlomoides
Salvia pichinchensis
Salvia pilosa M.Vahl=*Salvia rhombifolia* Ruiz & Pav.

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Salvia pinguifolia Rock Sage
Salvia pinnata
Salvia pisidica
Salvia pitcheri
Salvia plebeia R.Brown
Salvia plumosa Ruiz & Pav.
Salvia pogonocalyx Hance [Syn. *Salvia miltiorrhiza*]
Salvia pomifera Apple-bearing Sage
Salvia potentillifolia
Salvia praeclara Epling
Salvia pratensis Prairie-Meadow Sage, Meadow Clary, Meadow Sage
Salvia pratensis L.
Salvia prionitis
Salvia procumbens Ruiz & Pav.=*Salvia occidentalis* Sw.
Salvia przewalskii Maxim.
Salvia pseudococcinea Jacq.
Salvia pseudorosmarinus Epling
Salvia psilantha Epling
Salvia psilostachya Epling
Salvia puberula
Salvia punctata Ruiz & Pav. [Syn. *Salvia punctata* Ruiz & Pav.]
Salvia punctata Ruiz & Pav. [Syn: *Salvia punctata* Epling]
Salvia punctata van *glabra* Epling=*Salvia punctata* Ruiz & Pav.
Salvia pustulata Benth.=*Salvia bullulata* Benth.
Salvia radula Epling=*Salvia styphelus* Epling
Salvia reflexa Lance-Leaved Sage
Salvia reflexa Rocky Mountain Sage
Salvia regeliana
Salvia regla Mountain Sage
Salvia repens
Salvia revoluta Ruiz & Pav. [Syn. *Salvia revoluta* Epling]
Salvia rhodostephana Epling [Syn. *Salvia rhodostephana* Epling]
Salvia rhombifolia Ruiz & Pav. [Syn: *Salvia rhombifolia* M. Vahl]
Salvia rhombifolia van *glabrior* Benth.=*Salvia paposana* Philippi
Salvia rhyacophila
Salvia rigosa (rugosa?)
Salvia riparia Kunth=*Salvia misella* Kunth
Salvia rivularis Gardner
Salvia roemeriana Cedar Sage
Salvia rubescens Kunth
Salvia rubrifaux Epling
Salvia rufula
Salvia rugosa
Salvia runcinata
Salvia rusbyi Britton
Salvia sagittata Ruiz & Pav.

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Salvia saheudica
Salvia salvatrix Narrow-leaved White Sage, Sage “the Saviour”
Salvia salviaphilos
Salvia santolinifolia
Salvia sapinae
Salvia sarmentosa Epling
Salvia scabiosifolia
Salvia scandens Epling
Salvia sclarea Clary, Cleareye, Salvia, Clarry, Christ’s Eye, Clary Sage, Clear Eye, Common Clary, Eyebright, Garden Clary, Orvale, See Bright, Muskatellisalvia, Muscatel Sage, Tout-bonne, Clary Wort, Horminum, Gallitricum, Muskateller Salbei.
Salvia sclarea L.
Salvia sclareoides
Salvia sclareopsis
Salvia scrobiculata Meyen ex Bentham
=*Salvia tubiflora* Ruiz & Pav.
Salvia scutellarioides Kunth [Syn: *Salvia scutellarioides* Kunth]
Salvia serotina
Salvia sideritidis C.Presl ex Benth.=*Salvia griseifolia* Epling
Salvia silvarum Epling
Salvia sisymbrifolia
Salvia smyrnaea
Salvia somaliensis
Salvia sonomensis
Salvia spathacea Pitcher Sage
Salvia speciosa C.Presl ex Benth. [Syn. *Salvia speciosa* Benth.]
Salvia sphaceloides
Salvia spinosa
Salvia splendens Scarlet Sage
Salvia splendens Ker-Gawler
Salvia splendens Sellow ex Roem. & Schult.
Salvia squalens Kunth
Salvia stachydifolia Benth.
Salvia stenophylla
Salvia stepposa
Salvia striata Benth. [Syn. *Salvia striata* Briq.]
Salvia strictiflora Hooker=*Salvia oppositiflora* Ruiz & Pav.
Salvia styphelus Epling [Syn: *Salvia styphelus* Epling]
Salvia subincisa Saw-Tooth Sage
Salvia subscandens Epling & Jativa
Salvia summa Great Sage
Salvia syriaca
Salvia tafallae Benth.
Salvia tanshen Max. [Syn. *Salvia miltiorrbiza*]
Salvia tenella
Salvia tesquicola

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Salvia texana
Salvia tianscbanica
Salvia tiliaefolia
Salvia tiliifolia Vahl
Salvia tingitana
Salvia tomentosa Mill.
Salvia tortuosa
Salvia trichoclada
Salvia trifilis Epling
Salvia triloba Spanish Sage
Salvia tubiflora Ruiz & Pav. [Syn. *Salvia tubiflora* Meyen ex Bentham]
Salvia tubiflora Ruiz & Pav. [Syn: *Salvia tubiflora* Benth.]
Salvia tubulosa Epling
Salvia tuerckheimii
Salvia umbratica Epling=*Salvia obumbrata* Epling
Salvia uribei
Salvia urticifolia Nettle-Leaf Sage
Salvia urticifolia kasvaa Pohjois-Amerikassa
Salvia uruapana
Salvia vargasii Epling
Salvia vaseyi
Salvia verbenaca L.Oculus Christi, Vervain Sage,
Wild English Clary, Wild Clary
Salvia verbenacea
Salvia verticillata Lilac Sage
Salvia vestita Benth.
Salvia virgata Jacq.
Salvia viridis Painted Sage
Salvia viridis Red-topped Sage
Salvia viridis Salvia Bluebeard
Salvia weberbaueri Epling
Salvia willeana
Salvia xanthocheila
Salvia xanthophylla Epling & Jativa
Salvia yunnanensis C.H.Wright

SPECIFIC SPECIES OF SALVIA

Salvia officinalis

Pharmacopoeial status

Salvia officinalis is in the B.P.C. 1934, and the Pharmacopoeias of Austria, Czechoslovakia, Germany, Hungary, Jugoslavia, Netherlands, Poland, Portugal, Roumania, Russia and Switzerland [Martindale 1967].

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Planetary domination

It is under the domination of the planet Jupiter and symbolises domestic virtue [Leyel 1987].

The derivation of the name

The name of the genus, *Salvia,* is derived from the Latin *salvere,* to save, in reference to the curative properties of the plant, which was in olden times celebrated, as a medicinal herb. This name was corrupted popularly to Sauja and Sauge (the French form), in Old English, "Sawge', which has become our present-day name of Sage. [Grieve 1984].

History

Sage has been an important medicinal plant since earliest times. This is a herb that has the reputation as one which wards off evil. It was thought to be efficacious against the biting of serpents and the dispelling of evil spirits [Ceres 1984]. It was employed in ancient Egypt to increase the fertility of women [Schauenberg and Paris 1990]. It appears that sage was brought from ancient Egypt to our shores by the Romans [Onlooker 1995].

Theophrastus records two sages, one a spineless wild undershrub whose name he gives as Joakos (sphakos), the other resembling it, but cultivated, called (elelisphakos) [Theophrastus 1918]. Pliny the Elder says that this latter plant is called Salvia by the Romans, a mint-like, hoary and aromatic and also cultivated more than sphakos of Theophrastus, and used as a diuretic, for promoting menstruation, as a local anaesthetic (numbing the surface of the skin where it is applied), a styptic, and when taken in drink with wormwood, a treatment for dysentery [Pliny]. Monastery gardens in the time of the Carolingian empire of the early Middle Ages were cultivating the plant. Walahfrid Strabao, in his Hortulus, describes it as having a sweet scent and being of proved value in many human ailments, and he goes back to the Greek root for the name he gives it, Lelifagus [Strabao 1966]. There can be little doubt that, from the time of Theophrastus and Pliny on, the sage cultivated under these different names is *Salvia officinalis*.

An Anglo-Saxon manuscript reads "why should man die when he has sage?" A similar saying exists later since, the Salerno Medical School (11th and 12th centuries) wrote "Why should a Man die, if sage grows in his garden?—No garden medicament can prevail against the power of Death." The treatise closes with the words "Sage, thou healer, Nature's mediatrix".

Among the Ancients and throughout the Middle Ages it was in high repute: *Cur moriatur homo cui Salvia crescit in horto?* ('Why should a man die whilst sage grows in his garden?') has a corresponding English proverb: "He that would live for aye, Must eat Sage in May." [Grieve 1984]. This compares well with one from Germany, which has many proverbs about sage "for a ripe old age, in May you eat sage".

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The herb is sometimes spoken of as *S.salvatrix* ('Sage the Saviour'). An old tradition recommends that Rue shall be planted among the Sage, so as to keep away noxious toads from the valued and cherished plants. It was held that this plant would thrive or wither, just as the owner's business prospered or failed, and in Buckinghamshire, another tradition maintained that the wife rules when Sage grows vigorously in the garden [Grieve 1984].

Sage was one of the ingredients of Four Thieves Vinegar, a blend of herbs believed to protect the user against the plague. It was supposedly created around 1630 by four robbers from Toulouse who waited for plague victims to be removed for burial, and then entered their houses and looted them. They were eventually caught and sentenced to death, but so great was the need to find protection against the plague, that they were given their freedom in exchange for the recipe, which apparently left them untouched by the disease. It consisted of thyme, sage, lavender, rosemary and other herbs [Dragoco 1996].

In the Jura district of France, in Franche-Comte, the herb is supposed to mitigate grief, mental and bodily, and Pepys in his Diary for April 26th, 1661 said when travelling from Gosport to Southampton: "In our way…we observed a little churchyard, where the graves are accustomed to be strewed with sage" [Grieve 1984].

The following is a translation of an old French saying: "Sage helps the nerves and by its powerful might, Palsy is cured and fever put to flight," [Grieve, 1984]. A possible reference to Gerard, who recorded: "Sage is singularly good for the head and brain, it quickeneth the senses and memory, strengtheneth the sinews, restoreth health to those that have the palsy, and taketh away shakey trembling of the members" [Gerard 1990].

Dioscorides, Pliny and Galen all recommend sage as a haemostatic, diuretic, tonic and emmenagogue. Pliny tells us that it is good for cleansing snakebite.

Walafridus Strabo sings the praises of sage in his gardening book "Hortulus" and Charlemagne in the "Capitulare" decrees that sage must be cultivated on every farm. Folklore also said that that sage would make women fertile and arouse love for a person.

Country women would take sage to church with them, and if they got sleepy would have a sniff to wake them up!

In the United States Pharmacopceia, the leaves are still officially prescribed, as they were formerly in the London Pharmacopceia, but in Europe generally, Sage is now neglected by the regular medical practitioner, though is still used in domestic medicine.

Traditional uses of sage

In small doses it is anti-inflammatory.

Perspiration and fever

It is antihydrotic, and it reduces perspiration when taken as a tea, the action starts about 2 hours after drinking an effect which can last for several days [Fluck 1988;

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Leung 1989; Lust 1986]. This may be achieved by depressing fever control centre in the brain, as well as by relieving spasm in the smooth skeletal muscle [Winter-Griffith 1988]. New Orleans Blacks with either Cajun or Creole blood mixtures or both kinds in them, have used sage to reduce perspiration. Sage seems to have a calming effect on their sweat glands and effectively reduces outbreaks of sweat whether they occur in the underarm area, on hands, feet, or the entire body. A tea made of either the dried or fresh leaves and one cup of the same drank each day in small doses quickly controls excessive sweating [Ayensu 1981].

Oral preparations

It has value as a carminative, spasmolytic, antiseptic, astringent, and is used in a variety of complaints, the most relevant being inflammation of the mouth, tongue and throat, as a gargle or mouthwash [British Herbal Pharmacopoeia 1983]. It will soothe the soothes of the mucous membrane and be of benefit for inflamed and bleeding gums and good for mouth ulcers [Hoffman 1987]. It has been cited for use in bad breath [Buchmann 1987], It contains bactericidal principles. It is useful for sore throat [Leung 1989] and good results have been seen with peritonsillar abscesses as sage will give subjective relief and promote healing (gargle needs to be hot and repeated every 2 hours) [Weiss 1986]. It is even said to be able to regulate the flow of saliva [Bunney 1984]. The fact that infusions of the herb are effective mouth washes and gargles in househol medicine indicates fairly good bactericidal value in the oil [Arctander I960]. Use of the leaves, liquefied, to treat laryngitis, is mentioned in China [Flora Yunnanica 1977:3].

It is also used to make a tooth-powder (stain remover). Sage leaves and common sea salt are blended together and baked until hard, then the aggregated mixture is ground down and used as a tooth powder (Baïracli Levy 1991).

Indigestion

Sage has some value in relieving indigestion with gas or spasmodic pain [Evans 1989].

Cautions

Avoid during pregnancy since it stimulates the muscles of the uterus. Large doses are toxic [Fluck 1988; Mills 1989]. Sage should not be taken in large doses for a long period because of the thujone it contains [Leung 1989], this is said to be neurotoxic (Talalaj and Czechowicz 1989). The volatile oil is said to be a violent epileptiform convulsant, resembling the essential oils of absinthe and nutmeg [British Pharmaceutical Codex 1923].

Sexual debility

Phelps-Brown (1993) said in 1875 "It is called by some a most capital remedy for spermatorrhoea, and for excessive veneral desire, and I am one of those who know

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from experience in my practice that it is grand for what is termed sexual debility when its use is indicated."

Skin care

For large pores, sage can be of benefit as a compress or infusion. It can be used for similar purpose as a face pack. Sage cream can be used for cold sores near the mouth [Back 1987]. Elderly blacks living in Michigan have utilised crushed fresh sage leaves to get rid of warts on the face, neck, throat, hands and arms. An herbal wash of the same fresh leaves has been used to relieve bumps, sores, wounds, cuts and other skin injuries [Boyd 1984].

Sage and rosemary are two herbs which some American Blacks in various Southern States consider to be "soul cosmetics" just as they regard different kinds of food as being "soul food" [Watt and Breyer-Brandwijk 1962].

Bathing and washing

It is an antifungal. It is also used in baths to treat skin problems. [Stuart 1986]. Another source gives the following external properties, astringent, healing (cicatrising), antiseptic, tonic, antirheumatic in baths, for atonic wounds, sores, ulcers, dermatosis (eczemas) [Valnet 1986].

Rheumatism

Good embrocation for use in cases of rheumatism [Grieve 1984]. Sage embrocation is helpful for easing muscular pain, for sciatica and for loosening stiff and painful joints [Back 1987].

Wound treatment

Because of the tannin content it is astringent and anti-inflammatory. It is also used as a lotion or compress for wounds. [Fluck 1988], It is also an excellent lotion for ulcers and to heal raw abrasions of the skin [Grieve 1984].

Varicose veins and leg conditions

The use of sage has been recommended for varicose veins and leg ulcers [Trattler 1985], as well as warts on the legs [Buchmann 1987].

Hair care

The common sage is one of the best plants for darkening and toning hair. An infusion of the fresh leaves or tops is used [Genders 1985]. It has also been popularly used to darken the hair when applied to the scalp [Grieve 1984]. It is used in cases of alopecia [Valnet 1986]. Sage is a good hair tonic and the infusion, used as a hair lotion, can be rubbed on to the scalp every other day to ensure healthy shining hair. It is particularly

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good for dark hair, strengthening the hair and deepening the natural colour [Back 1987]. A mixture of garden sage and rosemary has been used by some of the Elderly blacks living in Michigan in the past to maintain the sheen of their dark, curly hair and to strengthen and stimulate further hair growth, this is probably attributable to the volatile oil in the plant [Lewis 1977].

A recipe for hair tonic and setting lotion: Cut up a handful of sage leaves and tops and the same quantity of rosemary. Place in one pint of cold water and bring slowly to the boil. Simmer for three minutes (keeping covered). Remove from heat and allow to steep for three hours. Massage into scalp and hair every night. [Baïracli Levy 1991].

Insects

It has been used for wasp stings and insect bites [Valnet 1986; Leung 1980]. Dabbed onto insect bites it takes away the sting and the itch [Ceres 1984]. It is stated that the oils is used in insecticidal preparations. The volatile oile in this and other labiates is known to be obnoxious to insects and to reduce their presence in gardens, ot the parts of gardens, where these herbs are planted [Council of Scientific 1972:2].

Nervous conditions

It is also used in nervous conditions, trembling, depression, and vertigo [Lust 1986].

Mental conditions

The use of sage in age, now seems to have some technical corroboration. Workers at the Neurochemical Pathology Unit maintained by the MRC at Newcastle General Hospital have discovered evidence that Common Sage *(Salvia officinalis)* might prove useful in the struggle against Alzheimer's disease. The oil produced by this plant inhibits the activity of acetylcholinesterase, which may play a role in the loss of memory associated with the disease. In Alzheimer's disease the progressive deterioration of memory is associated with a fall in the brain concentration of acetylcholine. This may be brought about by excessive activity of esterase, and progressive memory loss can be slowed by some sufferers by administration of the antagonist tacrine [Onlooker 1995].

It is said to improve brain nourishment and is known as the "thinker's tea" [Mindell 1992].

Nursing mothers

Nursing mothers have used sage to stop the flow of milk [Leung 1980].

Feet and pedicular problems

A sage lotion made in large quantity can be used for a foot bath while it is still hot, for weary, sore and strained ankles and feet [Ceres 1984].

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Posology

According to Then, Lemberkovics, and Marczal (1996) dosing of *Salvia offidnalis* L is described in Erg.6.* as the following:

4–6 g of leaf
0.1–0.3 g/day of volatile oil
2.5–7.5 g/day of alcoholic extract
1.8–3.0 g/day of fluid extract

* Erganzungsbuch zur 6. Ausgabe des Deutschen Arzneibuches.

According to Grieve (1984) and confirmed by Wren (1958), when a more stimulating effect to the throat is desirable, the gargle may be made of equal quantities of vinegar and water, 1/2 pint of hot malt vinegar being poured on 1 oz. of leaves, adding 1/2 pint of cold water. The infusion when made for internal use can be made simply by pouring 1 pint of boiling water on to 1 oz. of the dried herb, the dose being from a wineglassful to half a teacupful, as often as required, but the old-fashioned way of making it is more elaborate and the result is a pleasant drink, cooling in fevers, and also a cleanser and purifier of the blood. Half an ounce of fresh Sage leaves, 1 oz. of sugar, the juice of 1 lemon, or 1/4 oz. of grated rind, are infused in a quart of boiling water and strained off after half an hour.

Buchmann (1987) steeps 2 tablespoons of leaves in a pint of boiling water for 7–10 minutes and points out that the infusion will become too bitter if steeped too long in boiling water. The pulped leaves of sage can be applied as a poultice to painful stings and bites.

Phelps Brown (1993) has a similar recipe in the section "Things for the Sick room", where he describes Sage tea as being dried leaves of sage, half an ounce; boiling water, one quart. Infuse for half an hour and strain; may add sugar if desired.

In the Merck Index (1940) the average dose is given as 4 g of the dried leaf.

The leaves may be administered in powder or as an infusion (1 in 20). The dose is 1–4 g [BPC 1923]. According to Mills (1989) the dosage of the dried leaves is 0.5–3 g three times per day.

The dose of the powdered leaves is from 1.3–1.95 g, the infusion made by macerating 1 oz. of the leaves in a pint of boiling water, of which 60 g may be administered at once [Wood and Bache 1883].

As a tonic, helpful for colds, coughs, influenza, sore throats, constipation, digestive disturbances and calming for nervous conditions. Take as many sage leaves as will loosely fill a 2 pint (1.14 L) saucepan; 3 lb (1.36 kg) sugar; 1 gal (4.5 L) water. [Law 1973].

Salvia bowleyana Dunn

The species is apparently confined to China, where it is found south of the Yangtze in the central and south-eastern provinces, at altitudes of 30–960 m, on hillsides, in woodland, and beside water [Iconographia 1974 No.5293].

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It is very similar to *S. militorrhiza* Bunge. Its name in Chinese means Southern miltiorrhiza. It is used in medicine in the same way, discriminating very slightly [Fu-chien 1982:1].

Salvia coccinea

In Mexico, the flowers rubbed onto the cheeks instead of rouge [Reis and Lipp 1982].

In South America several large plants are boiled in one gallon of water for 10 minutes and used warm to bathe varicosities, blood clots and congested blood [Arvigo 1993].

Salvia columbariae

It is an annual herb native to south-western USA and northern Mexico. The Spanish name “Chia” is also used to refer to other species of *Salvia* which are used in the same way. This is more commonly referred to as “Golden Chia”.

It was an important food plant for the native American tribes of south-western USA and northern Mexico, who believed that as little as a tablespoon of the seed would sustain a tribesman for 24 hours on a arduous march. It was also used in folk medicine for the treatment of diarrhoea.

The early Spanish settlers found it made one of the best poultices for the treatment of gunshot wounds, once ground and moistened.

It was also used to treat eye inflammation. The seed absorbs water to form a mucilaginous coating around itself and when the seed is placed under the eyelid this gelatinous coating removes any foreign body that may be causing irritation without causing damage itself [Fletcher 1991].

Salvia digitaloides Diels

The species is found in mountainous country in northern and central Yunnan, on grassy slopes or in coniferous woodland, at altitudes of 2.500–3.400 m. Seeds were sent to England by George Forrest [Cowan 1952], A variety occurs in south-western Sichuan.

The root is used in medicine, for the same purpose as that of *S. miltiorrhiza* Bunge, namely to stimulate the circulation, treat women’s diseases, clear extra-vasated blood, and regulate the senses, also to allay the pains of swellings and clear out pus. Recently, it has been found eminently successful for use in cardiovascular and coronary heart disorders [Flora Yunnanica 1977].

Salvia divinorum

The Mazatec Indians of Oaxaca used leaves of the plant *Salvia divinorum* as the basis of an infusion known as “poyomatli” or “pipilzintzintli” [Mann, 1989]. It has been used by them as a vision-inducing plant in ritual curing [Reisfield 1993].

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Salvia hispanica

Salvia hispanica was also known as Chia, which was so highly regarded by the Aztec tribes of Mexico that their rulers took this crop as annual tribute from the peoples of their vast empire. Today the seeds are ground and served in nutritious drinks in Mexico and South America. They are still extensively used by native Americans as a food source [Noll 1994].

Oil of chia was used as a base for face and body paints, and for paints used on wall murals, lacquerware and pottery, as well as in in manuscripts with decorative or ceramic stamps. The Aztec words chiactic, chiaoacaio and chiauizaio refer to oily or greasy skin.

The seeds were used by the Aztecs to produce an oil they called chiamatl, which was mixed with an insect fat called aje (or axin as the Aztecs called it) as well as vegetable and mineral pigments to produce a highly coloured salve. This brightly coloured face and body paint was used by both men and women to denote their position in society.

The flowers were used by both sexes often in the form of ointment or pommade, where oil of chia was used for its emollient properties.

In medicine, chia was used to stimulate saliva, to relieve pain of the knees, for injured feet, as a lotion for stricken patients and for eruptions of the skin. Combined with a white willow, it was a cure for intestinal disorders and fevers.

The Aztecs has extensive knowledge of many medicinal herbs and would use Chia (in the form of a porridge or gruel) as a soothing base for their infusions.

Chia seeds yield 25 to 30% extractable oil, which is rich in essential fatty acids. (20% linoleic acid, 60% linolenic acid). Chia meal, an ingredient potentially useful in facial scrubs, contains 20% protein and is high in amino acids (lysine, methione, cystine). Currently under study are chia's naturally occurring lipid antioxidants which provide oxidative stability. In water chia seeds form a mucilage rich in polysaccharides and mucopolysaccharides [Wilson 1993].

This desert plant has been shown to be an excellent source of antioxidants. A major portion of the antioxidant activity of oil-seed and oil-seed flowers and concentrates is attributable to flavonoid and hydroxylated cinnamic acids [Pratt 1992].

Salvia horminum

A south European species *Salvia horminum* or the red-topped sage has been used by putting the leaves and seed into the vat of fermenting wine to increase the inebriating quality of the liquor. An infusion of the leaves has been considered a good gargle for sore gums, and powdered makes a good snuff.

Salvia lavandulaefolia

Salvia lavandulaefolia or Spanish Sage is closely related to *Salvia officinalis* or Garden Sage. It grows wild in Spain and Southwest France. Spanish Sage contains a volatile oil composed of highly variable amounts of camphor (11–34%), cineole (18–35%), limonene (1–41%), camphene (5–30%), alpha-pinene (4–20%), betapinene

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(6–19), linalool, linalyl acetate, borneol and others. It is reported to have antimicrobial properties. It was non-irritating and non-sensitising to human skin and non phototoxic [Leung, 1980]. It is used as a perfume component and flavouring.

Salvia miltiorrhiza

[Syn. *S. pogonocalyx* Hence]

The species is widely distributed in northern China from eastern Gansu to the province of Shangdong, extending north to the southernmost provind of north-east China, Lioaning, and south to the Huai river area in the Middle Yangtze province of Hubei. It grows also in Japan.

The root of this plant is most often used to encourage tissue growth, to invigourate and nourish the blood, and reduce swellings. It is antibacterial, anti-fungal, vasodilator, good for burns, ringworm, acne, hair loss, itching and urticaria [Leung, 1980].

It is employed as a female tonic in amenorrhoea, metrorrhagia, gastralgia, mastitis. Dose: 5–10 g. [Keys, 1976].

Extracts from *Salvia miltiorrhiza* ("Danshen") are widely used in China to treat coronary artery disease, particularly angina pectoris and myocardial infarction [Guo-Qing Liu, 1995]. One of the components of this extract, Tanshinone IIA (TS IIA) is a coronary vasodilator and antiischemic agent. It also has seda tive and tranquilising effects and is employed in the treatment of neurasthenic insomnia (the component miltirone may contribute to the sedative effects of the extract).

The plants is a molluscicide reported to obtain up to 50% mortality [Kuo Yuang Hua, 1982].

Anti-microbial proterties are reported in the species. Extracts are said to be effective against drug-resistant *Staphylococcus,* the name of the species not being given. Dihydratanshinone I, hydroxytanshinone II-A, kryptotanshinone, methyl tanshinate, and tanshinone II-B are reported to be bacteriostatic against *Staphylococcus aureus* [Duke & Ayensu, 1985].

Medicinal use of the root in China goes back at least to the 1st millenium B.C. It is considered to be a tonic, employed specifically to stimulate the circulation and regulate the menses [Chiang-su, 1982], curtail uterine bleeding, and relieve abdominal pain, and relieve also depression and insomnia [Barefoot, 1978]. Current Chinese-language regional Floras state that it has recently been used with eminent success in the treatment of cardiovascular and coronary heart disorders [Flora Yunnanica, 1977]. Beneficial effect on the heart as a property of plant is a belief of long standing in China. Li Shih-chen in his 16th century Pen-ts'ao kang-mu, in describing the traditional functions of five shen or ginseng-named plants, states that the root of tan-shen *(S. miltiorrhiza)* operates upon the heart [Bretschneider, 1895].

In pharmacological activity, the constituent tanshinone is said to be anti-inflammatory in rats with infective arthritis [Duke & Ayensu, 1985].

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Salvia plebeia R.Brown

The species is widespread as a weed in southern and eastern Asia. In India it occurs throughout the plains, and up to 1 900 m, in the hills [Watt, 1893]. In China it occurs throughout the country, except in the north-west and Tibet [Iconographia, 1974 No. 3298], and in this part of Asia it extends to Korea. In the Philippines it occurs in Luzon, in and about towns at low altitudes [Merrill, 1923]. Confinement to these localities suggests that the species has been introduced at some early date, and has escaped into the wild. It is not known to be in medicinal use now in the Philippines. In the Indo-Chinese region it is found in Vietnam and Cambodia [Lecomte, 1927].

The whole herb contains flavones, homoplantagenin, hispidulin, nepetin, nepetin, nepetrin, and eupafolin and its 7-monoglucoside among them. It also contains protocatechuic acid, 4-hydroxyproprionic acid, volatile oil and saponin. Sterols are few, but there are several terpenes [Fu-chien, 1982:2].

The seeds contain 15% of a fatty oil [Flora Hainanica, 1977].

Anthelmintic properties are reported in the herb, both in India [Council of Scientific & Industrial Research, 1972] and Korea [Perry, 1980].

The plant is reported to be bacteriostatic against *Staphylococcus aureus, Aspergillus fumigatus,* Phyllody disease, and *Pseudomonas* [Fu-chien, 1982:2].

The oil from the seeds can contribute to the manufacture of soap in China [Flora Hainanica, 1977]. In India, the mucilaginous properties of this oil cause it to be used to anoint women's hair, hold it in place, and keep it glossy [Watt, 1893].

Pharmacological action of the plant, in experiments with rats is reported as antitussive, antiasthmatic, and anti-inflammatory, when used in decoction, and as a cure for bronchitis [Fu-chien, 1982:2].

Medicinal use of the seeds, and of the other above-ground parts of the plant, are distinct. In the Indo-Chinese region of plant provides a tisane against stomach-ache [Lecomte, 1927], and the whole plants and the flowers are reportedly prescribed to treat cholic, cholera, and dysentery [Perry]. In India the leaves are said to relieve toothache; further, the herb is employed there as a diuretic and astringent [Fu-chien, 1982:2].

The mucilaginous seeds have long been used in native medicine in that country to treat gonorrhoea and menorrhagia [Watt, 1893], to which conditions [Chopra, 1958] adds diarrhoea and haemorrhoids. In China [Chiang-su, 1982:2] the plant is used as a febrifuge, detoxifier, diuretic, blood cooler, haemostatic, and for the reduction of swellings. It relieves painful swellings, bleeding piles and inflammation of the mammary gland. A decoction taken hot is used to treat tonsillitis, haemorrhage in pulmonary consumption, and sluggish blood developing slight erythema.

Salvia pomifera

Salvia pomifera, the applebearing sage, has a very peculiar growth and is common on some of the Greek islands. It has firm, fleshy protuberances which are about 2 cm thick and swell out from the branches of the plant. They are produced in the same manner as oak apples by the puncture of an insect of the *Cynips* genus. These

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excrescences are semi-transparent like jelly and are called Sage Apples, which is the name by which they are sold in the market. They are candied with sugar and made into a kind of sweetmeat and conserve which is highly regarded by the Greeks as a delicacy (also said to possess healing and salutary qualities). It has an agreeable and astringent flavour. This plant is considerably larger than the common garden sage and it has a flavour and more powerful smell, which is a cross between lavender and sage. It grows very abundantly to the size of a small shrub in Candia (Crete) and Syros. The leaves are collected annually, dried and used medicinally as an infusion. The Greeks are particular as to the time and manner in which they are collected, the date being May 1, before sunrise. The infusion produces profuse perspiration, languor, and even faintness if used to excess. There is a smaller Greek salvia in Greece, the *Salvia candica,* which does not have excrescences.

Salvia repens, Salvia rugosa, Salvia runcinata, and *Salvia sisymbrifolia*

Salvia repens, Salvia rugosa, Salvia runcinata, and *Salvia sisymbrifolia*—have all been used at various times by different Southeastern African tribes for treating bed sores, herpes lesions, stinging nettle rash, and swellings due to insect or mosquito bites and wasp stings. They are all used as decoctions, teas or simple lotions. Sometimes milk would be used to steep the sage in, with excellent results.

Salvia sclarea

The herb is antispasmodic and balsamic in nature and has been used both fresh and dry for digestive difficulties as a stomachic. It has also been employed in kidney disease with good results. The mucilage of the seeds has been used in ophthalmic disorders and a decoction of the herb was considered by herbalists to be efficacious in any complaint of the eyes [Wren, 1994].

Mucilage of the seeds is used in tumours [Leung, 1980].

Cold extract of clary will help draw out thorns and splinters and reduce inflammation. The dried roots, crushed and powdered, can be used like snuff to clear the head and ease a headache. An ointment made with clary leaves will help draw out inflammation and bring boils and spots to a head [Back, 1987].

This herb was first brought into use by the Germans who added it to Elderflowers and then infused them and added the liquid to the Rheinish wine which then became a Muscatel [Grieve, 1984].

It was used by the native Jamaicans, who considered it cooling and cleansing for ulcers, and who also used it for inflammation of the eyes. A decoction of the leaves boiled in coconut oil was considered beneficial for the stings of scorpions [Grieve, 1984].

The oil has come under increasing attention for its use in aromatherapy. It is said to act on the brain's thalamus similarly to the oils of grapefruit, rose and jasmine. The same action also helps relieve anxiety states, including those involving fear, paranoia and delusions [Holmes, 1993]. These properties have been examined scientifically [Stanassova-Shopova, 1970].

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It is a good relaxing oil with euphoric effect on sensitive people and may help with insomnia [Hoffmann, 1991]. It is also described as uplifting, especially if feeling weepy [Tisserand, 1985].

The oil is also used for its antidepressant, antiphlogistic, antiseptic, anti-spasmodic, astringent, carminative and deodorant properties. These properties make it useful in the treatment of boils and infections as well as useful in skin care. In cosmetics and toiletries, a clary sage bath is warming and very relaxing. Externally it cools inflammation, and is much used in skin care because of its scent. It is useful for inflamed, normal or over-hydrated skin [Tisserand, 1987].

It is particularly valued for inflamed and mature skins [Price, 1987].

Except being moderately irritating to rabbit skin, available data indicate the oil to be generally non-toxic [Leung, 1980]. Used as a fragrance component. It is a flavouring in the food industry. After the essential oil is removed the crude material is a source of sclareol which is converted to the sclareolide; both are used to flavour tobacco. Sclareolide is also used in the production of an ambergris substitute.

Salvia serotina

Salvia serotina is used in the form of either a tea or lotion for treating scratches, eczema, rash, itching, cuts, and burns on the skin by native blacks throughout the West Indies.

Salvia verbenaca

Salvia verbenaca or Wild Clary has properties similar to Garden Clary, but the wild variety is considered to be more potent. The seed is mucilaginous, and the mucilage was used to soothe the eye [Potterton, 1983]. In another reference the mucilage is used to cleanse the eye [Grieve, 1984].

Salvia yunnanensis C.H.Wright

[Syn. *S. bodinieri* Vaniot; *S. esquirolii* Lévl.]

The species goes under various book-names and local names in China, many of then having Lake Tien miltiorrhiza [Iconographia 1974 No. 5292], Lake Tien being the major lake in the eastern Yunnan, near Kunming, and among other names in Yunnan Small miltiorrhiza and Purple miltiorrhiza.

The species appears to be confined to south-western Provinces of China, where it occurs in south-western Sichuan, Guizhou in the western part, and from the north of Yunnan, through such central districts as Dali, to the south-easst of the province. The type was obtained from Mengtsz, not so far from the Vietnam border.

A national Flora [Iconographia 1974 No. 5292] states succinctly that the root is used for that of *S. miltiorrhgiza* Bunge. In Yunnan, recorded uses of the root in medicine lie in stimulating the circulation, regulating the menses, clearing fresh extravasated blood, stopping pain, and claming the spirits by lowering the effects of

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stress, [Flora Yunnanica 1977:2], all of them properties attributed also to *S. miltiorrhiza*.

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II. BOTANY
2. THE SAGE PLANTS OF GREECE: DISTRIBUTION AND INFRASPECIFIC VARIATION

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The vernacular name "sage" is attributed to different aromatic species of the genus *Salvia* L. which are widely used as spices, as well as in the food, drug and fragrance industry (cf. Lawrence 1979–1995 and references therein; Kokkini 1994). The genus *Salvia,* one of the largest Labiatae genera, includes c. 900 species and has an almost cosmopolitan distribution. SW and C Asia is considered its major centre of origin (Hedge 1992).

THE GENUS *SALVIA* IN GREECE

The Greek flora includes 23 taxa of the genus *Salvia*. With respect to their total native range (Hedge 1972, 1982; Greuter *et al.,* 1986), they can be distinguished as following:

(i) Five taxa have a very narrow range, growing only in Greece (*S. eichleriana* Halácsy, *S. pomifera* L. subsp. *pomifera* and *S. teddii* Turrill), or in Greece and Turkey (*S. napifolia* Jacq., *S. pomifera* L. subsp. *calycina* (Sm.) Hayek)

(ii) Three taxa are mainly found in the Balkan Peninsula and are extended either in Asia Minor (*S. amplexicaulis* Lam., *S. forskahlii* L.), or in Italy (*S. officinalis* L.)

(iii) Two taxa have a wider distribution in the Mediterranean area (*S. argentea* L., *S. fruticosa* Miller)

(iv) Thirteen taxa have a widespread occurrence in the Old World, whereas several of them have been introduced and naturalized in America and/or Australia (*S. aethiopis* L., *S. candidissima* Vahl, *S. glutinosa* L., *S. pinnata* L., *S. pratensis* L., *S. ringens* Sm., *S. sclarea* L., *S. sylvestris* L., *S. tomentosa* Miller, *S. verbenaca* L., *S. verticillata* L. subsp. *verticillata, S. virgata* Jacq. and S. *viridis* L.).

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THE SAGE PLANTS OF GREECE

Sage plants have been known for their medicinal properties since ancient times. Hippokrates (5th c BC), Theophrastus (4th c BC) and Dioscorides (1st c AD) referred to them with the names "elelisfakon" and "sfakon". Though many of their annotators have tried to interpret the species identity, the descriptions given could not define any particular species (Doganis 1969; Rivera *et al.,* 1994). In modern Greece, three *Salvia* species, named "faskomilo" or "alisfakia", are used as spices or as ingredients of folk remedies: (i) *S. fruticosa* (Fig. 1), which is known in the international trade as "Greek sage", (ii) *S. officinalis* (Fig. 2), the commercially known "Dalmatian or garden sage", and (iii) *S. pomifera* (Fig. 3), the "Cretan sage". The essential oil of *S. fruticosa* is a folk remedy for toothaches and intestinal complaints (Fragaki 1969; Vokou *et al.,* 1993; Rivera *et al.,* 1994). Recent studies have revealed that a leaf infusion of *S. fruticosa* has a hypoglycemic effect (Perfumi *et al.,* 1991); furthermore it has been shown that its essential oil exhibits antibacterial, cytotoxic and antiviral activities (Sivropoulou *et al.,* 1997).

The three sage species are strongly aromatic, many branched shrubs and reach heights up to 1.60 m. *S. fruticosa* and *S. officinalis,* members of the Section *Salvia,* have actinomorphic calyces with 5 equal or subequal teeth or bilabiate calyces with a 3-toothed upper lip and a 2-toothed lower lip. *S. pomifera* belongs to the Section *Hymenosphace* Bentham and is clearly distinguished from the other two species by its membranous-reticulate, bilabiate calyces. The upper lip of these calyces is entire, sinuate or shortly three-mucronate, whereas the lobes of the lower lip are obtuse, emarginate or mucronate (cf. Hedge 1972, 1982). Glandular trichomes, where the essential oils are secreted and accumulated, appear in all aerial parts of the three *Salvia* species. Two types of glandular trichomes are found: (a) capitate glandular hairs consisting of a unicellular, bicellular or multicellular stalk and a unicellular or bicellular secretory head, and (b) peltate glandular hairs consisting of a unicellular stalk and a multicellular (up to 12-celled) head (Verzar-Petri and Then 1975; Gupta and Bambie 1980; Bini Maleci *et al.,* 1983; Venkatachalam *et al.,* 1984; Werker *et al.,* 1985; Corsi and Corsi 1988; Werker 1993; Länger 1997). Galls are frequently formed on the stems of *S. fruticosa* and *S. pomifera* (Fig. 4). The three *Salvia* species have the same chromosome number, 2n=14 (Fedorov 1969; Bothmer 1970; Kuštrak *et al.,* 1986).

DISTRIBUTION OF THE SAGE PLANTS

Salvia fruticosa Miller (Greek sage)

S. fruticosa is an endemic species of the Eastern Mediterranean basin. Its total native range extends from Cyrenaica, Sicily and Southern Italy, through the Southern part of the Balkan peninsula to West Syria (Pignatti 1982; Hedge 1982; Greuter *et al.,* 1986) (Fig. 5). Furthermore, it is found as a naturalized plant in parts of the Western Mediterranean region *viz.* in Malta, Spain and Portugal (Greuter *et al.,* 1986). The species was probably introduced for cultivation in the Iberian peninsula by the

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Figure 1 *Salvia fruticosa*. (Drawn by F.L.Bauer, from Sibthorp and Smith, *Flora Graeca* 1(1), 1806).

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Figure 2 *Salvia officinalis.*

ancient Phoenicians and Greeks; remnants of these cultivations are found today in several coastal areas (Rivera *et al.,* 1994).

S. fruticosa is the most widespread sage species in Greece, forming extended populations in littoral areas of the mainland, as well as in the Ionian and Aegean

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Figure 3 *Salvia pomifera* subsp. *calycina*. (Drawn by F.L.Bauer, from Sibthorp and Smith, *Flora Graeca* 1(1), 1806).

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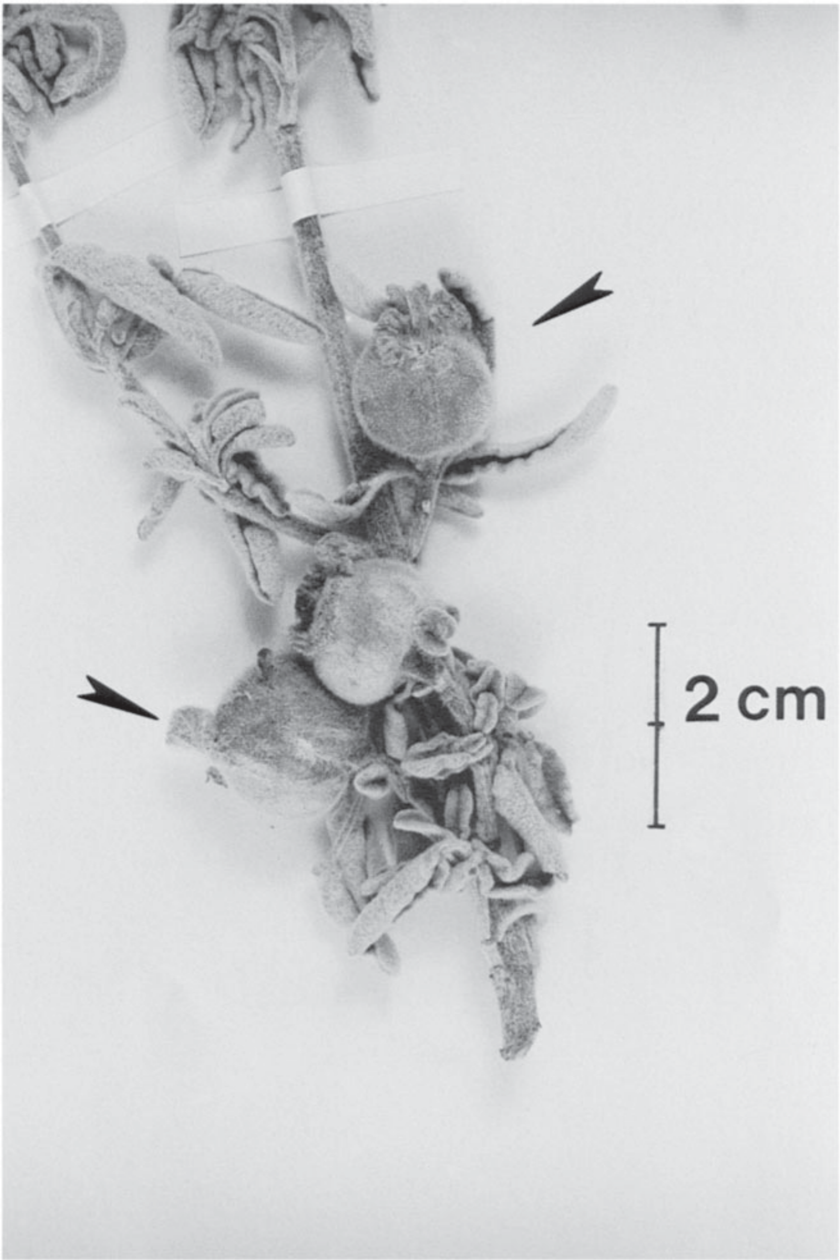

Figure 4 Gall formation in *Salvia fruticosa*.

islands, where macchies and phrygana dominate (Fig. 6). It grows at altitudes lower than 1000 m, rarely up to 1350 m. In the area of Southern Peloponnese and on several Aegean islands, it co-occurs with *S. pomifera*. The total range of *S. fruticosa*

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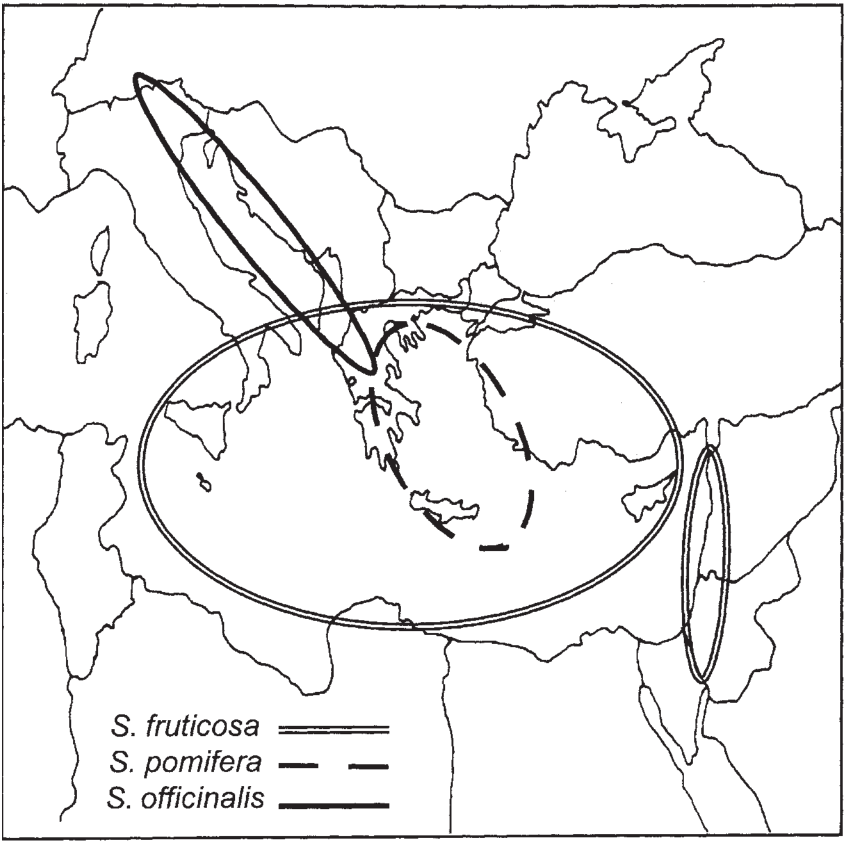

Figure 5 Schematic representation of the total distribution of the three sage species.

in Greece as well as that of *S. officinalis* and *S. pomifera* given in Figs 6, 7 and 8 is based on our own collections and literature data (Halácsy 1902, 1908; Rechinger 1943, 1949, 1961; Ganiatsas 1963; Greuter and Rechinger 1967; Boratynsky *et al.*, 1992).

Salvia officinalis L. (Dalmatian sage)

Its native distribution is restricted in the West Part of the Balkan Peninsula, i.e. in Albania, Former Jugoslavia, Greece as well as in northern Italy (Pignatti 1982; Greuter *et al.*, 1986) (Fig. 5). *S. officinalis* is naturalized in parts of S Europe (Greuter *et al.*, 1986). Furthermore, cultivated as a culinary herb or as an ornamen tal plant, it may be found all over Europe, where it was possibly introduced by Romans in ancient times or monks during the middle-ages (Gams 1927).

In Greece, *S. officinalis* grows only in the northwest part of the mainland at altitudes between 600 and 950 m. In particular, it is found in the shrublands of the

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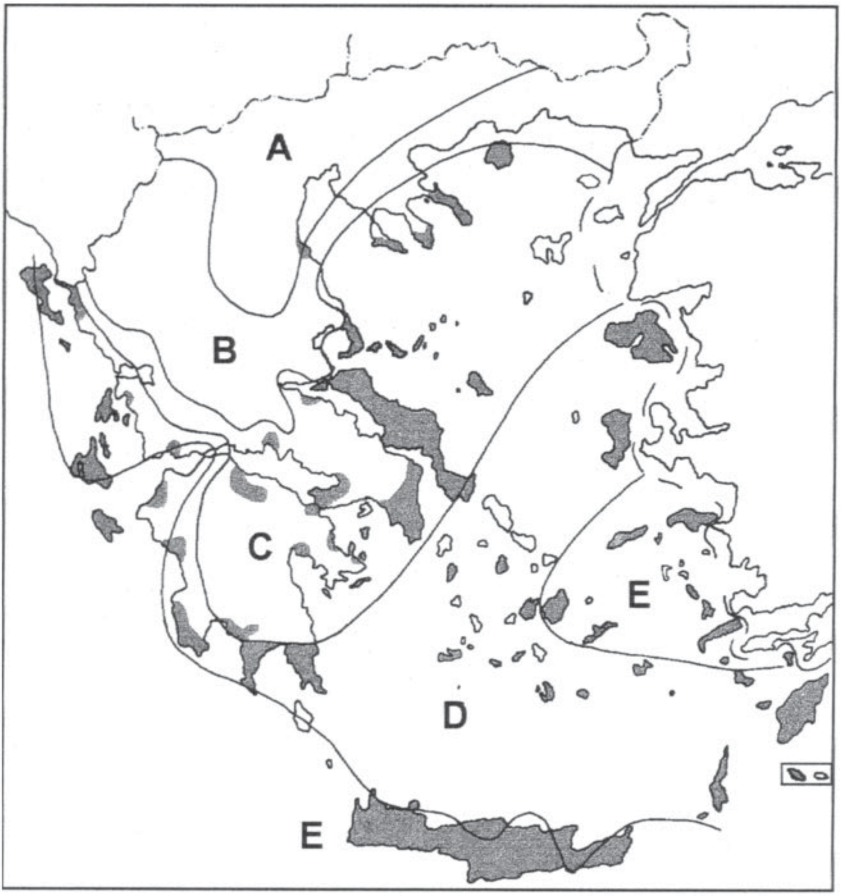

Figure 6 Distribution of *Salvia fruticosa* (Greek sage) in the five climatic zones of Greece. A: Continental Mediterranean zone. B: Transitional zone deviating to the Continental Mediterranean. C: Main transitional zone between the Continental Mediterranean and the Real Mediterranean zone. D: Real Mediterranean zone. E: Real Mediterranean zone with higher atmospheric stability than zone E (after Kotini-Zambaka 1983).

mixed deciduous zone of the mounts Voras, Pinovo, Vermion, Vourinos, Smolikas, Mitsikeli and Timfi. The species reaches its southernmost limit northwards the city of Arta (Fig. 7).

Salvia pomifera L. (Cretan sage)

The total range of *S. pomifera* is restricted in Greece and SW Anatolia (Hedge 1982; Greuter *et al.*, 1986). In Greece, it is found in parts of the SE mainland and the Aegean islands, at altitudes lower than 1350 m. It forms extended populations in the macchies and phrygana as well as in openings of pine forests (Fig. 8).

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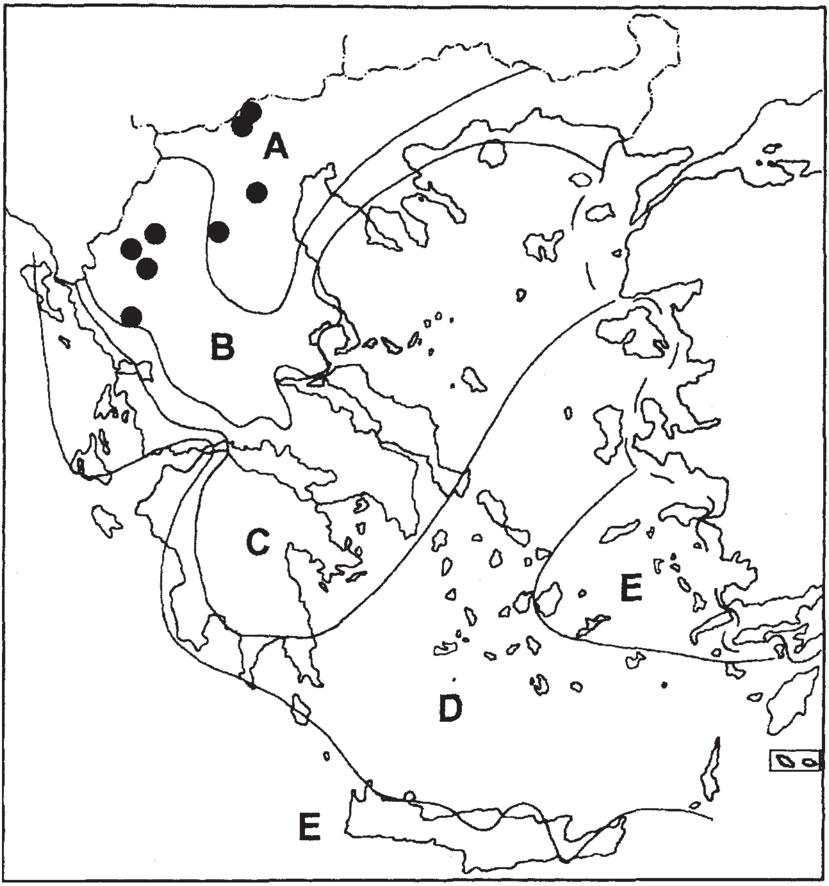

Figure 7 Distribution of *Salvia officinalis* (Greek sage) in the five climatic zones of Greece. (see Figure 6).

INFRASPECIFIC VARIATION OF THE SAGE PLANTS

Morphology

The Greek sage, *S. fruticosa,* was known until recently as *S. triloba* L. fil. (Hedge 1972, 1982). The specific epithet "*triloba*" (= three lobes) describes a species with three-lobed leaf blades. However, the Greek sage plants are very variable, having either entire or three-lobed leaves. This variation often causes confusion in the taxonomic identification of the wild growing plants. For example, *S. fruticosa* plants with entire leaves grown near the city of Kusadasi (W Anatolia) have recently described as a distinct species, informally named *Salvia "kusadasi"* (Marquard and Vomel 1983). Furthermore, *S. libanotica* Boiss & Gaill. in Boiss., *S. cypria* Kotschy and *S. lobryana* Aznav. have been described by older taxonomists as distinct species

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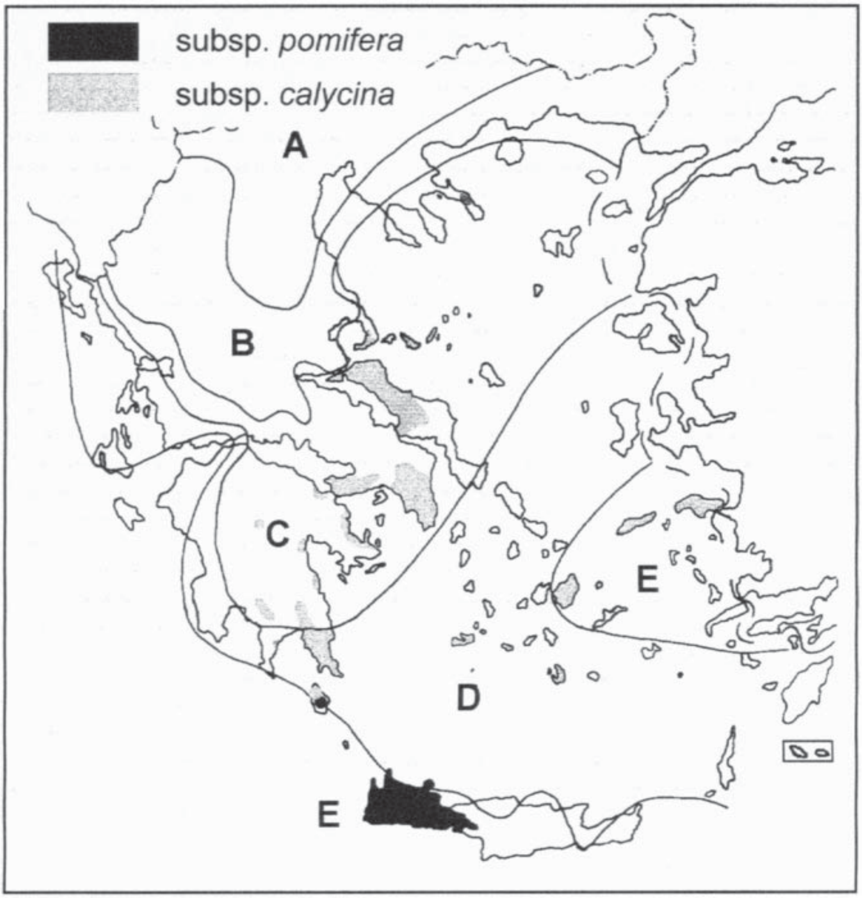

Figure 8 Distribution of *Salvia pomifera* (Cretan sage) in the five climatic zones of Greece (see Figure 6).

of the E Mediterranean area because of their small leaf size. These taxa are now considered as synonyms of *S. fruticosa* (cf. Greater *et al.*, 1986).

Collecting from the wild, one can easily realize that the leaf morphology of *S. fruticosa* highly varies in the different geographical areas. A characteristic pattern of *S. fruticosa* leaf variation is found along its native range on the island of Crete (Karousou 1995; Karousou and Kokkini 1997). *S. fruticosa* plants grown in the western part of Crete have entire leaves with flat blade and margins and dark green upper side (Fig. 9). Towards the eastern part of the island the leaves are much smaller, with deeply three-lobed, canaliculate, yellowish-green blade and strongly undulate margins (Fig. 9). It should be mentioned that along this direction (W→E Crete), the mean annual temperature and the total annual sunshine increases (Pennas 1977; Hager 1985; Egli 1993), whereas the mean annual precipitation decreases (Fig. 9).

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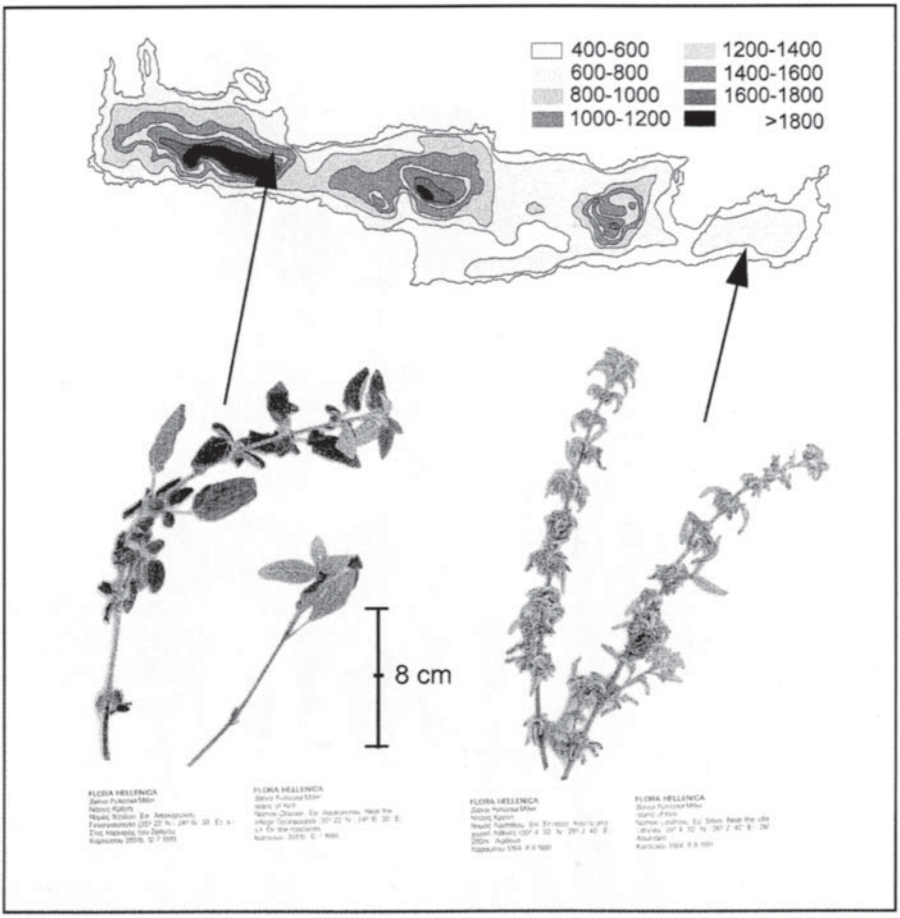

Figure 9 Map of the mean annual rainfall (in mm) of Crete (after Hager 1985) and representative *Salvia fruticosa* specimens from the western and eastern part of the island.

The climatically related variation of *S. fruticosa* leaf morphology is more profound when plants scattered along the total species range in Greece are considered. In the northern part of the country, where a Transitional climate between the Real Mediterranean and the Continental-Mediterranean occurs, *S. fruticosa* plants have flat and entire leaves. Towards S Greece, where a Real Mediterranean climate dominates, the total leaf surface decreases gradually and three-lobed, canaliculate-undulate forms appear (Fig. 10).

The effect of the different environmental conditions on *S. fruticosa* leaf morphology has been studied in a number of experiments by Szwarcbaum (1982). Plants cultivated in Israel under conditions of thermal and water stress exhibit relatively smaller and canaliculate leaves to overcome heat load. Measurements have shown that leaf temperature in canaliculate leaves is approximately 6 °C lower than in flat leaves. The canaliculate leaves expose to the sunshine their abaxial (lower)

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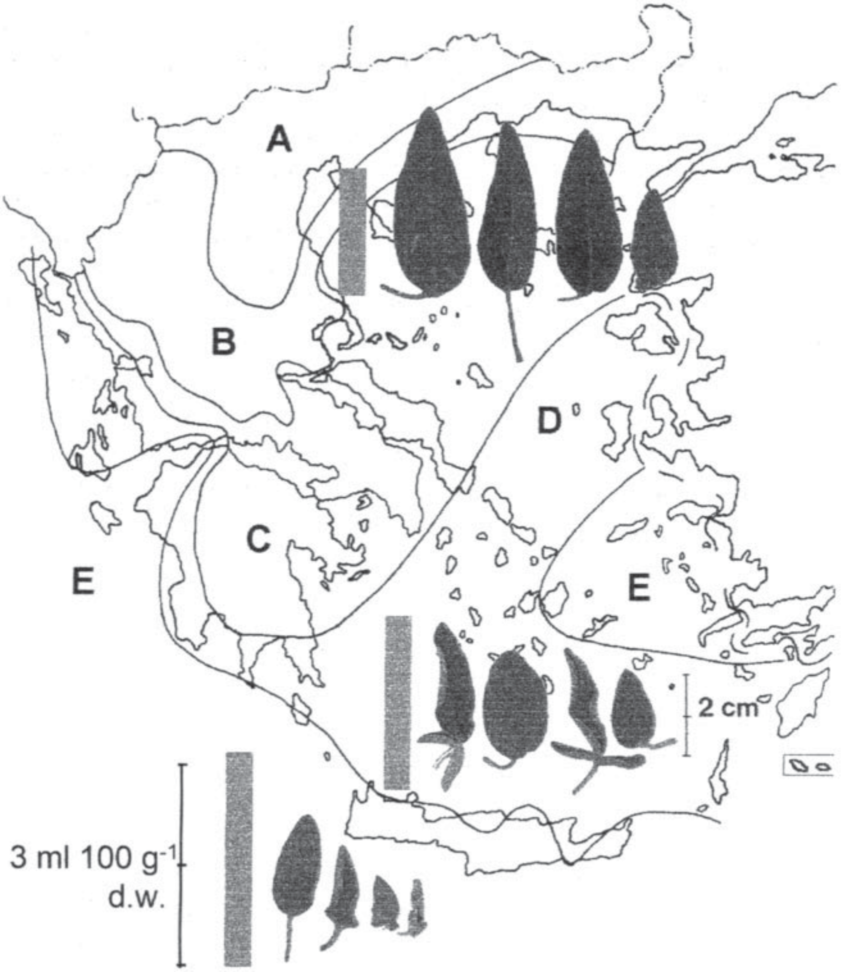

Figure 10 *Salvia fruticosa* (Greek sage). Infraspecific variation of leaf morphology and essential oil content. Representative leaves and mean values of essential oil content from different populations of each climatic zone.

face, which, due to a dense trichome layer, presents a higher reflectance in total incident solar radiation than the less hairy adaxial (upper) face. Similarly, the appearance of three-lobed leaves may also be considered as an adaptation to the xerothermic conditions, since the leaf dissection results to the lowering of the boundary layer resistance, the increase of the diffusion of water vapour and, consequently, to the increase of the heat flow from the leaf face (Fitter and Hay 1987 and references therein).

The Cretan sage, *S. pomifera,* is also a highly variable species. The specific epithet "*pomifera*" had been probably given to describe the frequent formation of galls (pommes) on the stems. Earlier taxonomists, based on leaf and calyx differences

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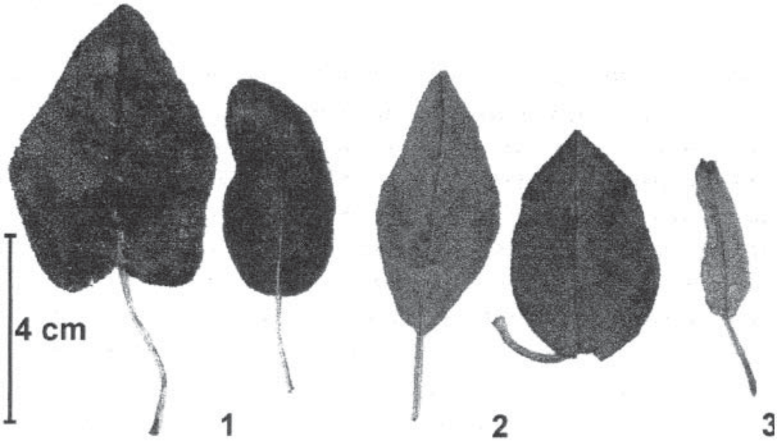

Figure 11 *Salvia pomifera* (Cretan sage). Infraspecific variation of leaf morphology. Representative leaves from different populations scattered all over Greece. 1: Athos peninsula; 2: Mt Parnitha (Attica); 3: Western Crete, near the village of Askyfou.

separated *S. pomifera* into two distinct species, *viz. S. pomifera* L. and *S. calycina* Sm. (Bentham 1848; Boissier 1879; Halácsy 1902; Rechinger 1943). More recently, Hedge (1982) synonymized these two species under *S. pomifera,* whereas in other accounts they were considered subspecies of *S. pomifera viz.* subsp. *pomifera* and subsp. *calycina* (Sm.) Hayek (Hayek 1931; Bothmer 1970; Greuter *et al.,* 1986). The two taxa are mainly distinguished by their leaf shape. Subsp. *pomifera* has oblong to linear-oblong, canaliculate-undulate, cuneate, rounded or subcordate at base leaves, whereas the leaves of subsp. *calycina* are broadly ovate to oblong, flat, rounded to cordate at base (Fig. 11).

S. pomifera subsp. *pomifera* is a Greek endemic taxon, restricted in the southern part of the species range. It grows only in the western part of the island of Crete and on the small island of Kythera (Fig. 8). *S. pomifera* subsp. *calycina* has a wider distribution and it is found in the eastern and south part of the Greek mainland the Aegean islands (Fig. 8) and in W Anatolia (Halácsy 1902, 1908; Rechinger 1943, 1949, 1961; Ganiatsas 1963; Bothmer 1970; Hedge 1982). Intermediate forms between the two subspecies have been mentioned from the island of Kythera and adjacent Peloponnese (Greuter and Rechinger 1967; Bothmer 1970).

In contrast to the highly variable Greek and Cretan sage, **Dalmatian sage** *(S. officinalis)* grown wild in Greece is fairly stable morphologically and can be distinguished by the oblong and always entire leaves (Fig. 12).

Essential Oils

Essential oil content

The essential oil content of the three sage plants in Greece shows a noticeable inter- and infraspecific variation. In particular it ranges from:

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Figure 12 Representative leaves of *Salvia officinalis* from Vikos gorge (NW Greece).

- 1.0 to 5.5% (ml 100 g^{-1} dry weight) in *S. fruticosa* (Catsiotis and Iconomou 1984; Harvala *et al.,* 1987; Kokkini *et al.,* 1989; Manou 1990; Karousou 1995; Karousou and Kokkini 1997; Karousou *et al.,* 1998a).
- 0.9 to 2.3% in *S. officinalis* (Kokkini *et al.,* 1989; Hanlidou 1996) and
- 1.3 to 4.2% in *S. pomifera* (Kokkini *et al.,* 1989; Bellomaria *et al.,* 1992; Skoula 1992; Karousou 1995; Karousou *et al.,* 1998b).

The variation observed within each species is both seasonal and geographical. Concerning seasonal variation, it has been found that the essential oil content varies following the annual fluctuations of the mean temperature and precipitation. The essential oil content of sage plants reaches the maximum values in summer and the minimum in winter and/or early spring (Manou 1990; Skoula 1992; Hanlidou 1996).

The study of different *S. fruticosa* populations collected during summer along the Greek territory revealed that their essential oil content decreases following the climatic gradient from the Real Mediterranean (zone E, mean value: 3.0%) to the Transitional Mediterranean climate (zone C, mean value: 1.9%). As can be seen in Fig. 10, the increase of the mean value of the essential oil content follows the changes in leaf morphology, i.e. the decrease of the total leaf surface and the formation of three-lobed, canaliculate-undulate blade.

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Qualitative and quantitative composition

The qualitative essential oil composition of the three *Salvia* species is similar with respect to the main components, 1, 8-cineole, α- + ß-thujone and camphor, which constitute the bulk of the oil (54.4–83.4%) (Catsiotis and Iconomou 1984; Harvala *et al.,* 1987; Bellomaria *et al.,* 1992; Skoula 1992; Karousou 1995; Hanlidou 1996; Karousou *et al.,* 1998a, b). However, a high inter- and infraspecific variation is found in the quantitative participation of the main components in the total oil (Table 1, Figs 13, 14). The amount of 1, 8-cineole is high in *S. fruticosa* oils, up to 66% of the total oil, whereas it is much lower, less than 16% in the other two sage species. The total thujone content is always high in *S. pomifera,* more than 58.7% of the total oil, whereas the highest amount of camphor (38.1%) has been recorded in *S. officinalis* oils. It should be noted that a total thujone content higher than 60.0% in a sage oil is rather rare and has been encountered only in a few *S. officinalis* oils (Lawrence 1979–1995 and references therein; Boelens and Boelens 1997 and references therein).

CONCLUSIONS

Three species of the genus *Salvia* are known as sage plants in Greece, *viz. S. fruticosa, S. officinalis* and *S. pomifera*. Their overall distribution is limited by the different climatic conditions dominating in the different areas of Greece. *S. officinalis* is restricted in the Continental-Mediterranean climatic zones (A and B), while *S.fruticosa* and *S. pomifera* occur only in the Real Mediterranean zones (D and E) and in the Transitional zone (C) between the Real Mediterranean and the Continental-Mediterranean climate.

S. fruticosa and *S. pomifera* exhibit a noticeable morphological variation along their range in Greece. In the latter species, this variation led to the recognition of two subspecies *viz.* subsp. *pomifera* and subsp. *calydna.* On the other hand, the leaf

Table 1 Ranges of the main essential oil components (% of the total oil) of the sage species in Greece.

	1,8-Cineole	*α- +β-Thujone*	*Camphor*	*References*
Salvia fruticosa	22.7–66.2	1.4–37.3	0.8–30.3	Catsiotis and Iconomou (1984); Harvala *et al.* (1987); Karousou (1995); Karousou *et al.* (1998a)
Salvia officinalis	13.4–15.4	15.8–32.7	30.3–38.1	Hanlidou (1996)
Salvia pomifera	0.2–9.5	58.7–83.0	0.3–3.8	Bellomaria *et al.* (1992); Skoula (1992); Karousou *et al.* (1998b)

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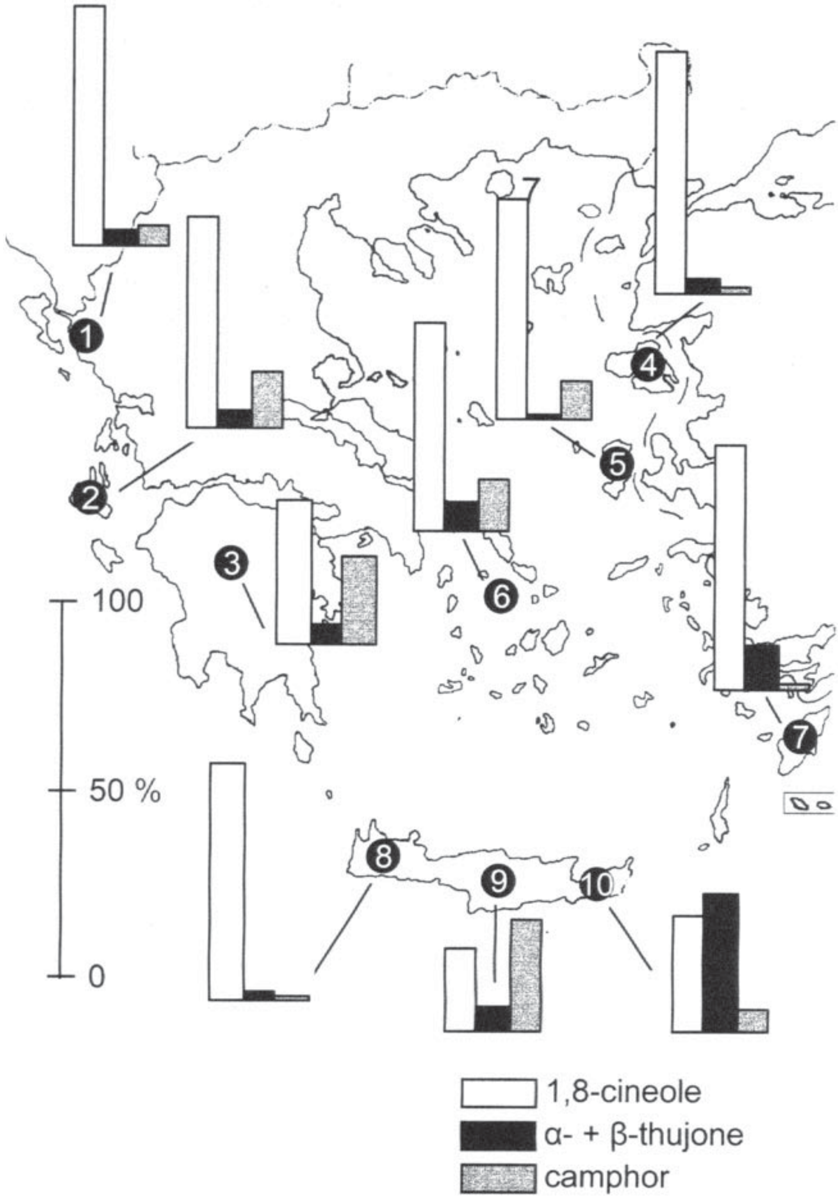

Figure 13 Infraspecific variation of the quantitative composition of *Salvia fruticosa* essential oils. The main components are expressed as percentages of the total oil. 1: Thesprotia; 2: island of Cephalonia; 3: Arkadia; 4: island of Lesvos; 5: island of Chios; 6: island of Syros; 7: island of Rodos; 8: Western Crete, Akrotiri Peninsula; 9: Central Crete, between the villages of Males and Christos; 10 Eastern Crete, near the village of Adravasti. The data for the localities are from Catsiotis and Iconomou (1984): loc. 1–7, Karousou *et al.* (1998a): loc. 8–10.

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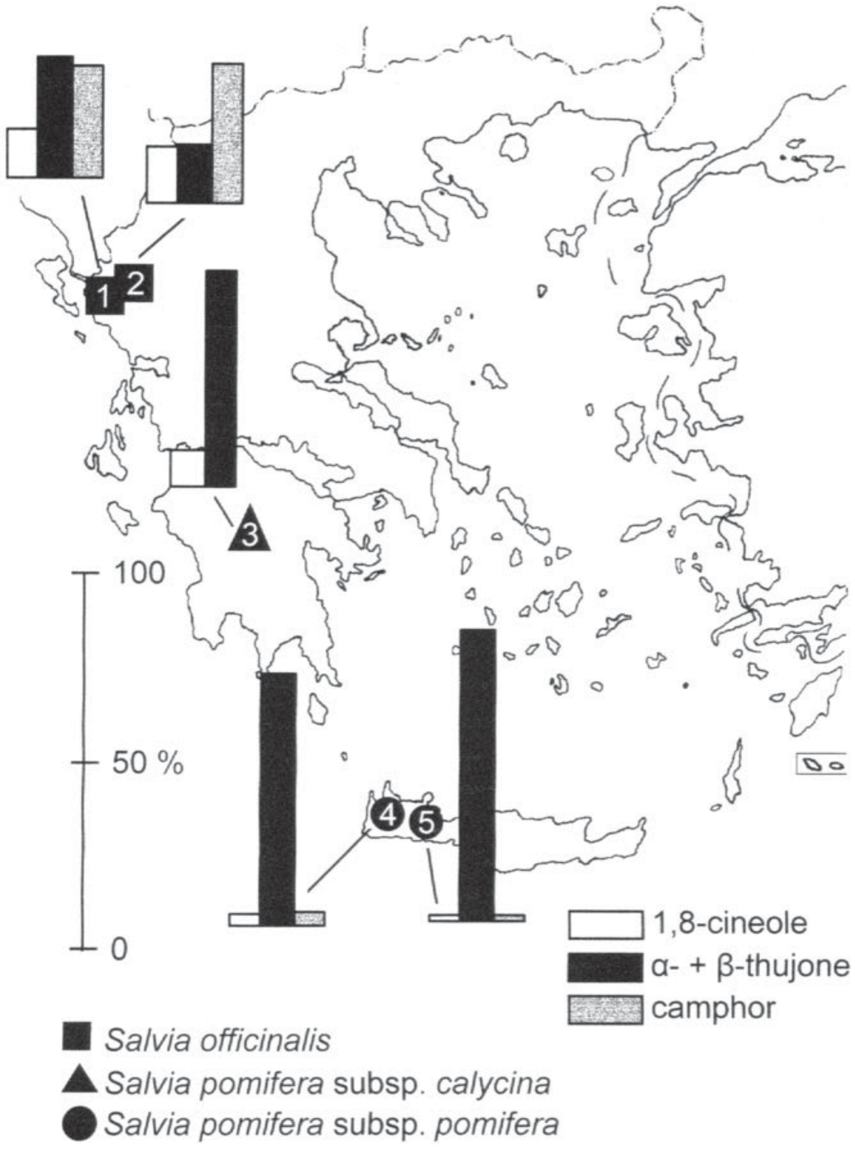

Figure 14 Infraspecific variation of the quantitative composition of *Salvia officinalis* and *S. pomifera* essential oils. The main components are expressed as percentages of the total oil. 1: Aoos gorge; 2: Vikos gorge; 3: Peloponnisos; 4: Western Crete, Sougia gorge; 5: Western Crete, Imbros Gorge. The data for the localities are from Hanlidou (1996): loc. 1–2, Bellomaria *et al.* (1992): loc. 3, and Karousou *et al.* (1998b): loc. 4-5.

morphology and the essential oil content of *S. fruticosa* plants change gradually following the geographic-climatic gradient along the species range in Greece. Plants from the northern part of the country have flat, simple leaves and a mean essential oil

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content less than 2.0%. Conversely, those grown in the southernmost areas of their distribution have smaller, often canaliculate-undulate, three-lobed leaves and a higher essential oil content (more than 2.9% in average). The essential oils of the three sage species are characterized by the same main components, *viz.* 1, 8-cineole, α- + ß-thujone and camphor, which constitute the bulk of the oil. A high variation in their quantitative participation is in particular found in the oils of Greek sage. These may be rich either in 1, 8-cineole, or in camphor, or in *α- + ß*-thujone. The high variation of both leaf morphology and essential oil features, should be taken into account when these characters are used for the taxonomic or commercial identification of the sage plants.

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3. *SALVIA* IN SOUTHERN AFRICA

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INTRODUCTION

Salvia species of Africa, particularly southern Africa, have not been very well studied. With regard to taxonomy, a revision of *Salvia* in Africa was carried out by Hedge (1974). The 59 species recognized were divided into 18 species-groups, of which the seven occur in southern Africa. The author, however, pointed out that further work on African *Salvia* is required, especially a comparison with other Old World species would be of value.

Most of the species are restricted to Africa. The distribution of the genus extends all over northern Africa from west to east and southwards to the east African highlands. The genus is absent from most of western and central tropical Africa. This means that the species in southern Africa are geographically isolated from the species on the northern part of the continent. Few species are common to both northern and southern Africa.

GEOGRAPHICAL DISTRIBUTION

Southern Africa is home to 30 species of the genus *Salvia*. The geographical distribution of the *Salvia* species growing in southern Africa is given in Table 1 (Arnold and de Wet 1993). Due to the size of South Africa and the different climatic regions within the country a breakdown in areas was done. These areas are The Cape, The Free State, Natal and Transvaal.

Most of the species only occur in sparse populations. A few species, *S. africana-lutea* and *S. chamelaeagnea* are, however, relatively common in the Knysna area of the Cape Province. Four of the species, *S. coccinea, S. reflexa, S. sclarea* (Clary Sage) and *S. tiliifolia* have been introduced into southern Africa, and are thus not endemic to the region. *S. verbenaca* is probably indigenous to the contries around the Mediterranean and on the Canary Islands and has spread further afield in Europe and Asia (Codd 1985). If it was introduced it is now widely distributed.

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Table 1 Geographical distribution of *Salvia* species in southern Africa.

Botanical name	*Geographical distribution*
S. africana-caerulea L.	The Cape
S. africana-lutea L.	The Cape
S. albicaulis Benth.	The Cape
S. aurita L. f. var. *aurita*	The Cape, Transvaal
S. aurita L. f. var. *galpinii* (Skan) Hedge	The Cape, Natal, Transvaal and Swaziland
S. chamelaeagnea Berg.	The Cape
S. coccinea Etlinger	The Cape, Natal, Transvaal, Namibia and Swaziland (Introduced)
S. dentata Ait.	The Cape
S. disermas L.	The Cape, the Free State and Transvaal
S. dolomitica Codd	Transvaal
S. garipensis E. Mey. ex Benth.	The Cape and Namibia
S. granitica Hochst.	The Cape
S. lanceolata Lam.	The Cape
S. muirii L. Bol.	The Cape
S. namaensis Schinz	The Cape, the Free State and Namibia
S. obtusata Thunb.	The Cape
S. radula Benth.	Transvaal
S. reflexa Hornem.	The Cape, The Free State, Transvaal and Lesotho (Introduced)
S. repens Burch. ex Benth. var *keiensis* Hedge	The Cape
S. repens Burch. ex Benth. var *repens*	The Cape, the Free State, Natal, Transvaal and Lesotho
S. repens Burch. ex Benth. var. *transvaalensis* Hedge	The Free State and Transvaal
S. runcinata L. f.	The Cape, the Free State, Natal, Transvaal and Lesotho
S. scabra L.f.	The Cape
S. schlechteri Briq.	The Cape
S. sclarea L.	(Introduced)
S. stenophylla Burch. ex Benth.	The Cape, the Free State, Natal, Transvaal, Namibia, Lesotho, Botswana
S. tiliifolia Vahl	Transvaal (Introduced)
S. triangularis Thunb.	The Cape
S. tysonii Skan	The Cape, Natal
S. verbenaca L.	The Cape, The Free State, Transvaal, Namibia and Lesotho

SALVIA IN TRADITIONAL MEDICINE

Traditional medicine plays a very important role in health care in southern Africa. In South Africa it is estimated that 80% of the black population consults with

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traditional healers. This situation is not likely to change in the foreseeable future as many patients have more faith in traditional healing than in Western-type health care, and might prefer traditional healing even when Western medicine is available. Traditional healers have centuries of experience in using plants for healing purposes. Traditional usages of *Salvia* species by different population groups are listed in Table 2. Only nine species, a third of the species occurring in southern Africa, have been used for medicinal purposes.

Very roughly, the people of South Africa can be divided into four ethnic groups, the Khoisan (formerly known as bushmen or hottentots), the black, the white and the coloured. These groups have to a more or lesser degree interacted on usage of medicinal plants. The Khoisan, coloured and white people have to a large extent used the same plants. The Khoisans had a very thorough knowledge of the veld and medicinal plants. They taught the coloured people working as farm labourers which plants to use for medicinal purposes. This knowledge was then absorbed by the white farmers (Anonymous 1993).

It is, however, very probable that the Europeans would have recognized various *Salvia* species. The Europeans utilized the local *Salvia* species for much the same ailments as *S. officinalis* is used for in Europe, mostly colds, coughs and chest trouble.

Various black tribes use extracts of *Salvia* species to treat infants. They also use the plants for a purpose none of the other groups do, namely to disinfect their huts.

CHEMICAL CONSTITUENTS IN *SALVIA* SPECIES

Very limited phytochemical work has been done on the *Salvia* species occurring in southern Africa. The chemistry of introduced species will not be covered there, as that will be dealt with in other chapters.

The essential oil of *S. stenophylla* collected in the High Veld of the Free State in South Africa was investigated by GC-MS (Jequier *et al.,* 1980) The volatile monoterpenes, expecially a-phellandren, were present in high concentration (28% of total oil). The oxygenated monoterpenoids constituted 5% of the oil, and the sesquiterpene hydrocarbons 35.5%. Among the oxygenated sesquiterpenoids which constitute the bulk of the oil (46%), a-bisabolol (41%) and manool (4%) were the most abundant. These compounds are mainly responsible for the persistent wood odour of *S. stenophylla. S. stenophylla* contains very low amounts of 1, 8-cineole and camphor, and lacked a- and b-thujone. a(R)- and (S)-Sinensal are unique constituents of *S. stenophylla.*

Brunke and Hammerschmidt (1985) analyzed the essential oil of *S. stenophylla* and identified 44 compounds. Most of the compounds were well known natural substances. The oil contained 29.8% a-bisabolol, which was isolated by column chromatography and identified as (+)-epi-a-bisabolol. It was the first time this isomer had been shown to occur in nature.

An exudate of *S. stenophylla* collected in the Eastern Cape was investigated for flavonoids (Wollenweber *et al.,* 1992). Apigenin, apigenin-7-methyl ether, scutellarein-7,4′-dimethyl ether, luteolin and 6-hydroxyluteolin-6, 7-dimethyl ether

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Table 2 Traditional usage of *Salvia* species in southern Africa.

Botanical name	*Traditional usage*	*References*
S. africana-caerulea	Used by European settlers as a remedy for coughs, colds and chest troubles, a tincture also for whooping cough and uterine troubles. An old household remedy for colic, diarrhoea, heartburn and indigestion prepared as a tea to which Epsom salt and lemon juice was added. Used by the Nama people for coughs, colds and female ailments. The Rastafarians use it for chest problems, colds, kidney infections, stomach trouble and women's ailments. Given to cows after calving to aid in the expulsion of the placenta.	Laidler 1928. Watt and Breyer-Brandwijk 1962. Dyson 1996.
S. africana-lutea	Used by European settlers for colds. The Nama use a decoction for coughs, colds and female ailments.	Laidler 1928. Dyson 1996.
S. chamelaeagnea	Used by European settlers for coughs, including whooping cough, colds and bronchitis, as well as for diarrhoea. The coloured population of the Cape use an infusion of the dry leaf for convulsions. The Nama use it for coughs, colds and female ailments. Used for burns, chest complains, flu and fever, and for head-,ear-and stomach pains.	Laidler 1928. Watt and Breyer-Brandwijk 1962. Anonymous 1993.
S. coccinea	Used by the European settlers for the relief of lumbago, "kidney disease" and the cough of pulmonary tuberculosis. Have caused livestock death.	Hurst 1942. Watt and Breyer-Brandwijk 1962. Hutchings *et al.* 1996.
S. disermas	Is used as a lotion for sores and as a tea. Used for the heart, high blood pressure and rheumatism.	Watt and Breyer-Brandwijk 1962. Anonymous 1993.
S. reflexa	Have caused livestock death.	Everist 1981.

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Table 2 Continued

Botanical name	*Traditional usage*	*References*
S. repens	Is added to the bath for treating sores on the body. The Southern Sotho take a decoction of the root before meals for stomach ache and diarrhoea, also used for diarrhoea in cattle. The Basuto use the smoke from burning the plant to disinfect a hut after sickness and to drive away bugs. They also mix the plant with their tobacco.	Phillips 1917. Watt and Breyer-Brandwijk 1962.
S. runcinata	A decoction of root, leaf and stem used by European settlers for the relief of urticaria. The Southern Sotho burn the plant in a hut to disinfect after sickness and mix it with their tobacco. The Zulu administer leaf paste purgatives to infants.	Phillips 1917. Watt and Breyer-Brandwijk 1962. Hutchings *et al.* 1996.
S. scabra	A paste of the leaf is made with mother's milk and given as the first medicine to Xhosa infants. A water extract of the roots is given daily for two months to newborns.	Smith 1888. Watt and Breyer-Brandwijk 1962.
S. sclarea	The coloured people of the Cape use a milk decoction for sore throat and a water decoction to treat sores and swellings. The Southern Sotho use decoctions of the root before meals for stomach ache and diarrhoea.	Watt and Breyer-Brandwijk 1962. Phillips 1917.
S. stenophylla	The Southern Sotho burn the plant in a hut to disinfect after sickness and mix it with their tobacco.	Phillips 1917.

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accumulated in the exudate. The main constituent was, however, an unidentified polar phenolic compound.

No work has been done on *S. verbenaca* plants growing in southern Africa. Apigenin, luteolin, salvigenin and 5-hydroxy 7′,4′-dimethoxy flavone has been isolated from leaves of *S. verbenaca* growing in Spain (Camarasa *et al.,* 1982). A root extract of Egyptian *S. verbenaca* plants contained taxodione, horminone and 6b-hydroxy-7a-acetoxyroyleanone (Sabri *et al.,* 1989).

A large study on the occurrence of alkaloids in plants also included a number of the southern African *Salvia* species. *S. chamelaeagmea, S. namaensis* and *S. runcinata* tested positive for alkaloids, whereas *S. africana caerulea, S. africana lutea, S. coccinea, S. dolomitica, S. reflexa, S. tiliifolia* and *S. triangularis* tested negative (Raffauf 1996).

INDUSTRIAL PRODUCTION

An essential oils industry is absent in South Africa in spite of the fact that southern Africa is richly endowed with aromatic plants. There is obviously potential for the development of such an industry considering the plant material present as well as the climate and relatively inexpensive labour force. At present an initiative is underway to establish a commercial production of essential oils in the Eastern Cape province. This initiative is a cooperation between universities, the Ministry of Agriculture and international funding agencies. It is hoped such an initiative can benefit especially small scale farmers as part of the land redistribution reform in South Africa.

Salvia stenophylla is one of the few known sources of epi-a-bisabolol, an anti-inflammatory agent which is fast becoming a very important additive for skin care products. Until recently the only feasible source of natural bisabolol was the essential oil of Camomile flowers, which contains approximately 17% of the active ingredient. As the oil of *S. stenophylla* contains around 30% bisabolol it may become an important crop. This species is at present undergoing field trials in the Eastern Cape.

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III. CHEMICAL CONSTITUENTS
4. TERPENOIDS IN THE GENUS *SALVIA*

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INTRODUCTION

The name *Salvia* came from Latin word Salvare (healer). Since ancient times *Salvia* species have had many uses as folk medicine. *Salvia* L. being one of the largest genera in the Labiatae family, is represented by 900 species in the world (Stanley and Williams 1973). Although *Salvia* species are common all around the world, they are abundantly located in three areas, in Europa around the Mediterranean, in South-East Asia, and in Central and South America.

A literature survey and our experiences indicated that the main components of *Salvia* species are flavonoids and terpenoids. The aerial parts of these plants usually contain flavonoids and triterpenoids as well as the volatile compounds, such as monoterpenoids, while in the roots, the main compounds are diterpenoids. However, American *Salvia* species contain diterpenes in the aerial parts. Both sesquiterpenoids and sesterterpenes are rather rare in *Salvia* species.

In this article the presence of terpenoids in *Salvia* species will be discussed, with the exception of volatile terpenoids (monoterpenes).

TRITERPENOIDS

Figures 1–18 show some of the triterpenoids. The most common triterpenoids, found almost in all *Salvia* species are ursolic (Fig. 1) and oleanolic (Fig. 2) acids isolated from *S. officinalis* L. (Brieskorn and Schlumprecht 1951). In later years other triterpenoids were isolated from *Salvia* species and their structures were established. As examples anagadiol (Fig. 3), taraxerol acetate (Fig. 4), germanicol (Fig. 5), *a*-amiradienyl acetate (Fig. 6) and nivadiol (Fig. 7) could be given, which were isolated from *S. broussonetti* Benth. by Gonzales *et al.* (1972). Our group has isolated a number of new triterpenoids from the aerial parts of Turkish *Salvia* species. Vergatic acid (Fig. 8) from *S. virgata* Jacq., (Ulubelen and Ayanoglu 1976), a tri-terpenic alcohol 2ß, 3ß-dihydroxyolean-13(18)-en (9) from *S. horminum* (Ulubelen *et al.*, 1977), 2ß, 3ß, 11α-trihydroxy olean-13(18)-en (Fig. 10) from *S. pinnata* L. (Ulubelen and Topçu 1984), 2ß, 3ß-dihydroxyolean-5, 12-dien-28-oic acid (Fig. 11), 2ß, 3ß, 18-trihydroxyolean-12-en-28-oic acid (Fig. 12) from S. *tomentosa* Mill., (Ulubelen and Topçu 1992), salvinemerol (Fig. 13) from *S. nemorosa* L. (Ulubelen *et al.*, 1994). Two new triterpenoids, przewanoic acids A (Fig 14) and B (Fig 15) were isolated from *S. przewalskii* (Wang *et al.*, 1988).

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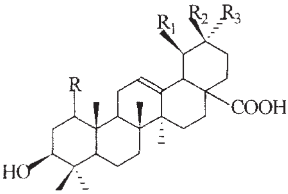
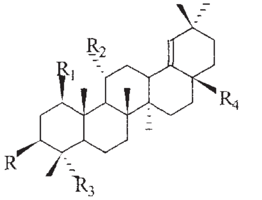


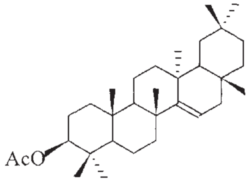
1 R=H, R_1=R_2=Me,R_3=H

2 R=R_1=H,R_2=R_3=Me

8 R=O,R_1=H,R_2=R_3=Me



	R	R_1	R_2	R_3	R_4
3	OH	OH	H	Me	Me
5	OH	H	H	Me	Me
7	OH	H	OH	Me	Me



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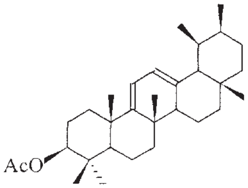

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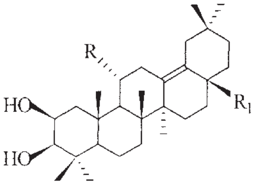
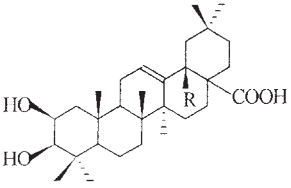


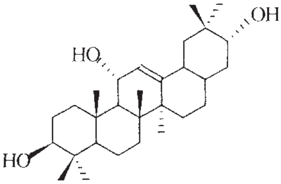
9 R=H, R_1=Me

10 R=OH, R_1=COOH



11 R=H $\Delta^{5,6}$

12 R=OH



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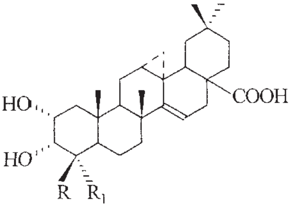
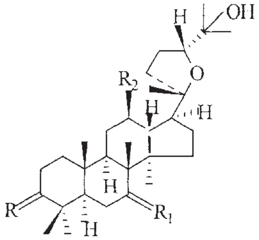


14 R=R_1=Me

15 R,R_1=CH_2



	R	R_1	R_2
16	O	αH,βOH	H
17	αH,βOH	αOH,βH	H
18	O	H	OH

Figures 1–18 Ursan, oleanane and dammarane type triterpenoids.

Dammarene-type triterpenoids are also present in the aerial parts of *Salvia* species. *S. bicolor* has yielded 20S, 24R-epoxydammar-12*ß*, 25-diol-3-one (Fig. 16) (Valverde *et al.,* 1985). Salvilymitone (Fig. 17) and salvilymitol (Fig. 18) were isolated from *S. hierosolymitana* (Pedreros *et al.,* 1990).

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DITERPENOIDS

Salvia species contain abietane, clerodane, pimarane and labdane-type diterpenoids. Except the American species, *Salvia* contains mostly abietane-type diterpenoids in their roots, clerodane and labdane diterpenoids being rather rare. In American species of *Salvia* clerodane diterpenes are found in their aerial parts or in the whole plants.

Abietane Diterpenoids

Tanshinones are quite well known abietane diterpenes, first isolated by Nakao from *S. miltiorrbiza* Bunge. according to Kakisawa *et al.* (1968). The first diterpenoids obtained from this plant were tanshinones I, II and III (Figs 19–21), later isotanshinones I and II (Figs 22, 23) and isocryptotanshinone (Fig. 24) and cryptotanshinone (Fig. 25) (Kakisawa *et al.,* 1969) were isolated.

Since *Salvia miltiorrbiza* showed a number of biological activities, the same type of compounds were searched for in other *Salvia* species. Among them cryptotanshinone (Fig. 25), 6-deoxo-5, 6-didehydrolanugon Q (Fig. 26) from *S. apiana* (Gonzales *et al.,* 1992), tanshinone II (Fig. 20) and 3*a*, 17-dihydroxytanshinone II (Fig. 27) from S. *hians* (Khetwal *et al.,* 1992). Quite a number of abietane diterpenes with aromatic or quinoid C-rings were isolated from *Salvia* species, many of which possessed biological activities. Two new compounds, aucatriol (Fig. 28) and galdosol (Fig. 29) were obtained from *S. canariensis* L. by Gonzales *et al.* (1975). A quinone methide (Fig. 30) together with carnosol (Fig. 31), carnosic acid (32), rosmanol (Fig. 33), isorosmanol (Fig. 34), galdasol (Fig. 29), isogaldasol (Fig, 35) and a rearranged abietane diterpene rosmadiol (Fig. 36) were found in *S. mellifera* by Gonzales *et al.* (1992).

A number of new abietane diterpenoids were isolated from Turkish *Salvia* species, most of them having biological activities. Hypargenins A-B (Figs 37–38) and D-F (Figs 39–41) show antibacterial activity, these compounds were isolated from *S. hypargeia* Fisch and Mey. (Ulubelen *et al.,* 1988) while an interesting abietane diterpenoid, wiedelactone (Fig. 42) was isolated from *S. wiedemannii* Boiss (Ulubelen *et al.,* 1991). Seven new diterpenoids, pomiferins A-G (Figs 43–49) from *S. pomifera* (Ulubelen and Topçu 1992), and five new abietane diterpenes from *S. napifolia* Jacq. 1-oxo-ferruginol (Fig. 50), 6-oxoferruginol (Fig. 51), 7, 20-epoxyroyleanone (Fig. 52), 11, 12-dioxo-abieta-8, 13-dien (Fig. 53), 6, 12, 14-tri-hydroxyabieta-6, 8, 11, 13-tetraen (Fig. 54) (Ulubelen *et al.,* 1995) were also obtained from various *Salvia* species.

Rearranged *Abietane Diterpenes*

Rearranged abietane diterpenoids were also obtained from *Salvia* species. Aethiopinone (Fig. 55) was isolated from *S. aethiopis* by Boya and Valverde (1981). Later salvipisone (Fig. 56) was obtained from the same plant, by Rodriguez *et al.* (1984). While from another plant, *S. argentea,* 1-oxo-aethiopinone (Fig. 57) was isolated by Michavila *et al.* (1986). *S. prionitis* has yielded some rearranged diterpenes, 4-hydroxysapriparaquinone (Fig. 58), sapriorthoquinone (Fig. 59) and a

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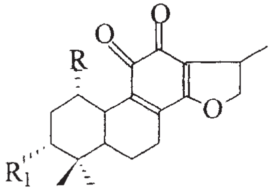
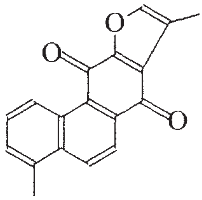


19 $R=R_1=H$, $\Delta^{5(10),6(7),15(16)}$
20 $R=R_1=H$, $\Delta^{5(10),6(7)}$
21 $R=R_1=OH$, $\Delta^{15(16)}$
25 $R=R_1=H$, $\Delta^{5(10),6(7)}$



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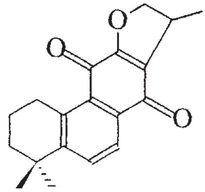
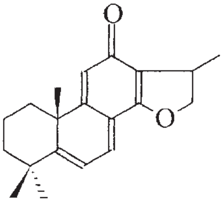


23 $\Delta^{15(16)}$
24



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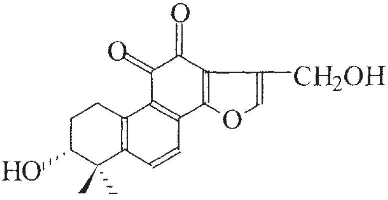

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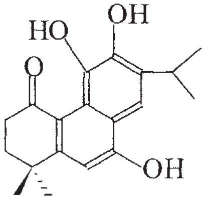

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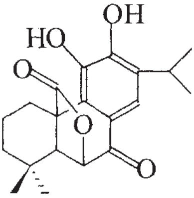

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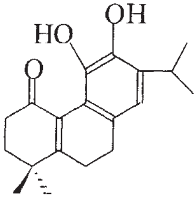

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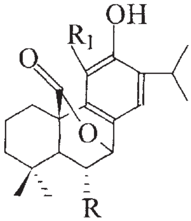
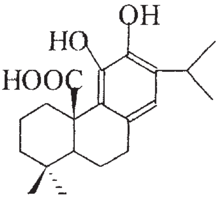


31 $R=H$, $R_1=OH$
34 $R=OH$, $R_1=O$
35 $R=O$, $R_1=H$



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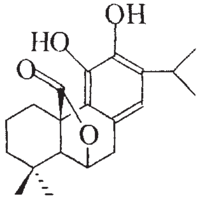

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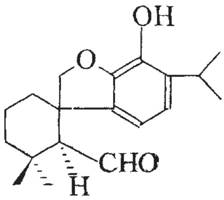

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Figures 19–36 Abietane type diterpenoids.

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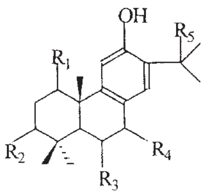
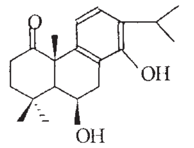


37 $R_1=R_4=O$, $R_2=R_5=H$, $R_3=\beta$ OH

38 $R_1=R_2=R_3=H$, $R_4=O$, $R_5=OH$

40 $R_1=R_3=R_4=R_5=H$, $R_2=O\Delta^{6,7}$



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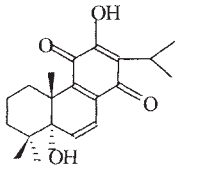

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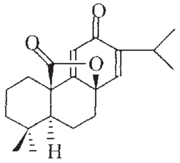

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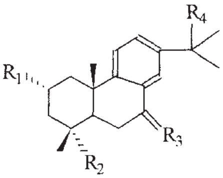
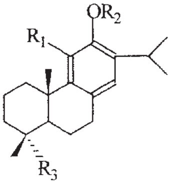
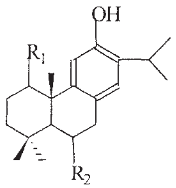


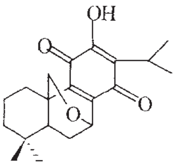
	R_1	R_2	R_3	R_4
43	H	CH_2OH	H	H
46	OH	Me	H	H
47	OH	Me	O	H
48	OH	Me	O	OH
49	H	CH_2OAc	O	H



	R_1	R_2	R_3
44	H	H	CH_2OAc
45	OH	Me	COOH



	R_1	R_2
50	O	H
51	H	O



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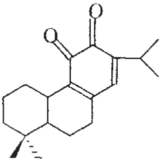

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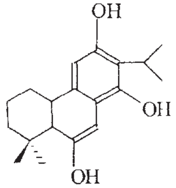

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Figures 37–54 Abietane type diterpenoids.

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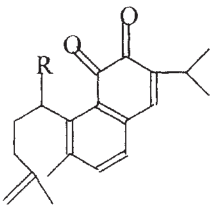
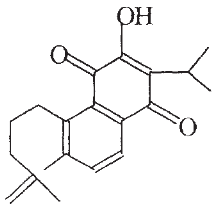


55 R=H

57 R=O



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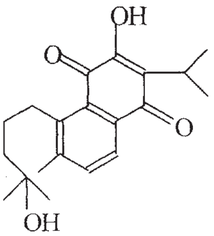

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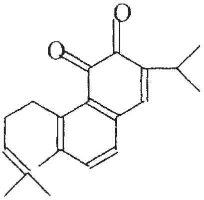

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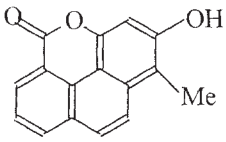

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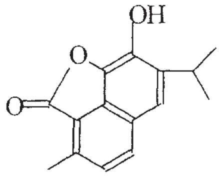

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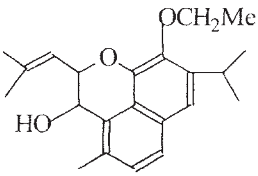

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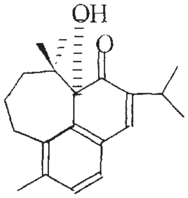

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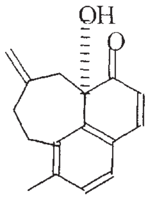

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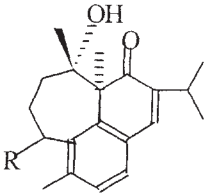

65 R=H

66 R=O

Figures 55–66 Rearranged abietane diterpenoids.

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new tetracyclic diterpene derivative salvinolactone (Fig. 60), a norditerpene with a new skeletal type, sapriolactone (Fig. 61) as well as salvonitin (Fig. 62) (Lin *et al.,* 1989 a, b, c). Microstegiol (Fig. 63) is an interesting rearranged abietane diterpene obtained from *S. microstegia* Boiss. The same type rearranged compounds candidissiol (Fig. 64) was isolated from *S. candidissima* Vahl (Ulubelen *et al.,* 1992 a, b). Salvibretol (65) and 1-oxo-salvibretol (Fig. 66) were isolated from *S. montbretii* Benth. (Topçu and Ulubelen 1996). There are of course many more rearranged abietane diterpenoids found in other *Salvia* species which have not been mentioned.

Clerodane Diterpenoids

American *Salvia* species contain mostly clerodane diterpenoids and rarely abietane type in the aerial parts or in the whole plant. Rodriguez-Hahn *et al.* (1973) isolated a clerodane diterpene, melisodoric acid, (Fig. 67) from a Mexican plant, *S. melissodara* Lag. and languiduline (Fig. 68) was obtained from *S. languidula* by the same group (Cardenas *et al.,* 1979). The structure of (Fig.. 68) was confirmed by X-ray analysis. From the aerial parts of another Mexican *Salvia* species, *S. fulgens,* a novel rearranged neo-clerodane diterpene, salvigenolide, (Fig. 69) was isolated by Esquivel *et al.* (1985). Semiatrin (Fig. 70) from *S. semiatratha* Zucc. and three new diterpenes from S. *lineata* Benth. 1(10)-dehydrosalviarin (Fig. 71) 1α, 10α-epoxysalviarin (Fig. 72) linearifoline (Fig. 73), as well as languiduline (Fig. 74) from *S. sousae* were isolated by Esquivel *et al.* (1986 a, b, 1988). Languiduline type diterpenoids are obtained from Mexican *Salvia* species. Recently, Maldonado and Ortega (1997) obtained tonalenin (Fig. 75) and another compound (Fig. 71) from *S.tonalensis.* Although there are a few studies by the European scientists on the structure of clerodane diterpenoids, collected from the botanical garden of Palermo in Italy, the origin of the plants was Mexico. (Savona *et al.,* 1982, 1983) have investigated *Salvia coccinea* and *S. farinacea* and found novel diterpenoids of clerodane-type, salvicoccin (Fig. 76) from the first, and salvifarin (Fig. 77) and salvifaricin (Fig. 78) from the second plant, in both cases the aerial parts of the plants were studied.

Labdane and *Pimarane* Type *Diterpenoids*

These type of diterpenoids are rather rare in *Salvia* species, although they are found in other Labiatae plants. Michavila *et al.* (1986 b) isolated sclareol (Fig. 79) and 6*a*-hydroxysclareol (Fig. 80) from *S. moorcrattiana.*

The aerial parts of *S.wiedemannii* has yielded 7*ß*-hydroxysandracopimaric acid (Fig. 81) and 14-oxopimaric acid (Fig. 82) together with abietane diterpenoids (Topçu and Ulubelen 1990, 1991). A study of S. *candidissima* subsp. *occidentalis* yielded manoyloxide (Fig. 83) (Ulubelen *et al.,* 1992 b). Three pimarane derivatives were isolated from *S. heldrichiana* Boiss. which is an endemic plant to eastern Mediterranean area, these are isopimaric acid (Fig. 84), 7*ß*-hydroxysandracopimaric acid (Fig. 81) and 7-oxo-13-*epi*-pimara-8,15-dien-18-oic acid (Fig. 85) (Ulubelen *et al.,* 1995). Recently we have isolated two pimarane diterpenes from *S. multicaulis* Vahl. Manool and a new compound salvipimarone (Fig. 86) which induced a mild

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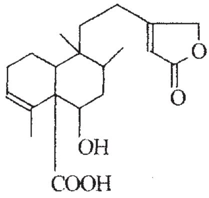

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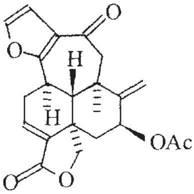

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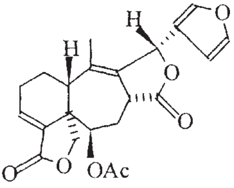

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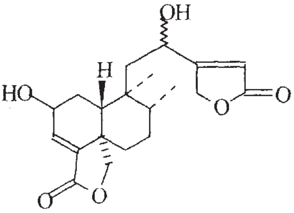

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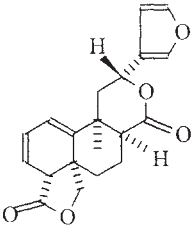

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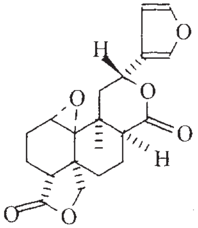

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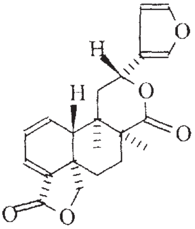

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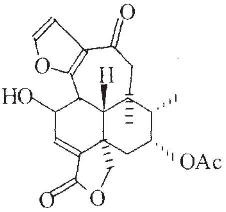

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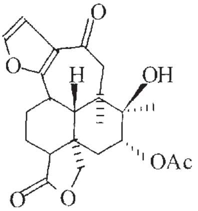

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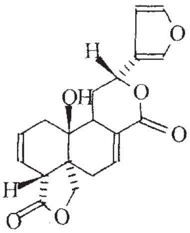

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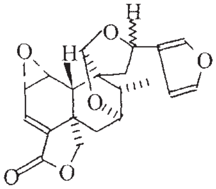

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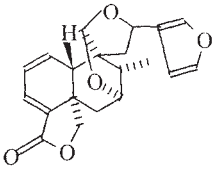

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Figures 67–78 Neoclerodane and rearranged neoclerodane diterpenoids.

activity against *Mycobacterium tuberculosis* as well as against *Proteus mirabilis* (ATCC 14153) and *Entrococcus faecalis* (ATCC 29212) (Ulubelen *et al.*, 1997).

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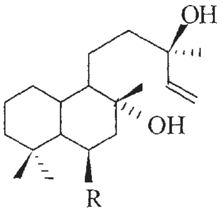
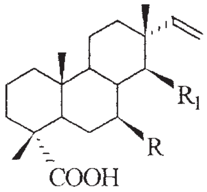


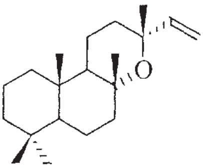
79 R=H

80 R=OH



81 R=OH, R_1=H, $\Delta^{8(14)}$

82 R=H, R_1=O



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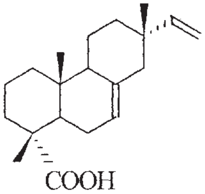

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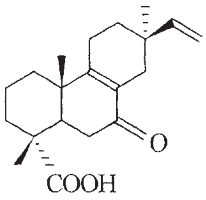

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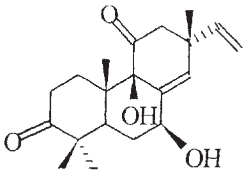

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Figures 79–86 Pimarane and labdane diterpenoids.

SESQUITERPENOIDS

Sesquiterpenoids are rare compounds in *Salvia* species. *S. palaefolia* H.B.K. a South America plant has yielded two sesquiterpenoids, caryophyllene oxide (Fig. 87) and glechomafuran (Fig. 88) (Gonzales *et al.,* 1989). In another study with the same plant material, the same group has isolated sesquiterpene lactones 8-hydroxyglechomanolide (Fig. 89) and two eudesmanolides, 1α-acetoxy-8α-hydroxy-8-hydroxy-2-oxo-eudesman-3, 7(11)-dien-8, 12-olide (Fig. 90) and its deacetyl derivative (Fig. 91) (Gonzales *et al.*, 1990). *S. yosgadensis* has yielded a sesquiterpene spathulenol (Fig. 92), and sesquiterpene lactones, Istanbulin D (Fig.

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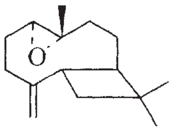

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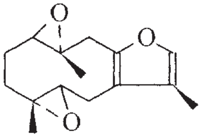

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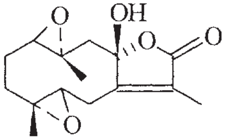

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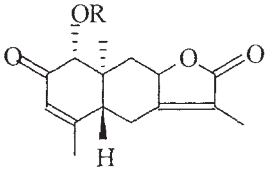
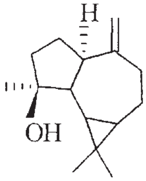


90 R=Ac

91 R=H



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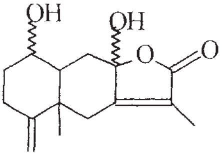

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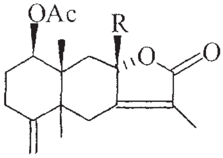

94 R=OH

95 R=H

Figures 87–95 Sesquiterpenoids.

93), 1ß-acetoxy-8ß-hydroxyeudesman-4(15), 7-dien-8, 12-olide (Fig. 94), 1ß-*ace*-toxyeudesm-8(15),7-dien-8, 12-olide (Fig. 95) (Topçu *et al.,* 1996).

SESTERTERPENOIDS

Rustaiyan *et al.* (1982, 1988) isolated sesterterpenes from *S. hypoleuca* Benth with a new skeleton, salvi -leucolide methyl ester (Fig. 96) and salvileucolide 6, 23-lactone (Fig. 97) as well as salvileucolide methyl ester derivatives (Figs 98, 99, 100, 101,) and

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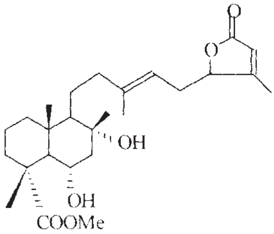

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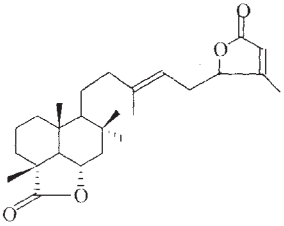

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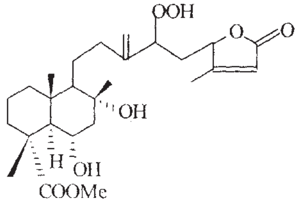

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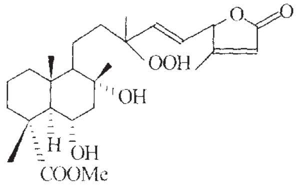

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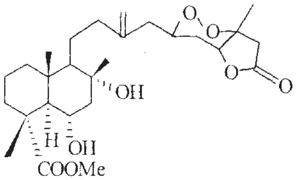

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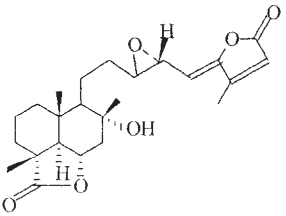

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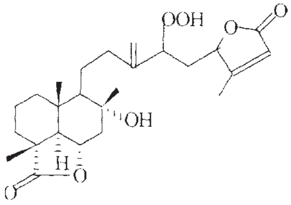

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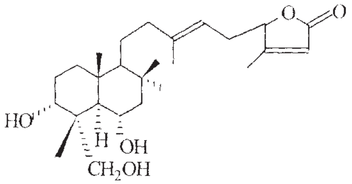

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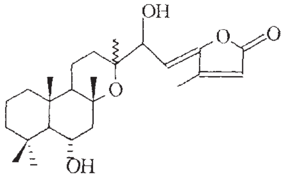

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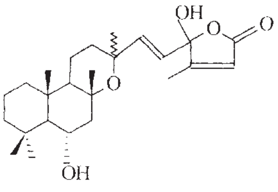

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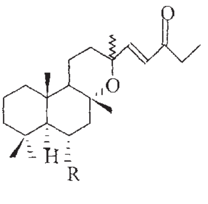

108 13β Me, R=OH
109 13α Me, R=OH
110 13β Me, R=H
111 13α Me, R=H

Figures 96–111 Sesterterpenoids.

isomeric epoxides (Figs 102, 103) and a hydroperoxide (Fig. 104). From another Iranian plant, *Salvia sahendica* Boiss and Buhse salvileucolide methyl ester (Fig. 96) and its 6, 23 lactone (Fig. 97) were also isolated (Moghaddam *et al.,* 1995).

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Rustaiyan and Sadjadi (1987) isolated sesterterpenes similar to salvileucolides, salvisyriacolide (Fig. 105) from *S. syriaca* L.

Two new sesterterpene lactones, yosgadensolide A (Fig. 106) and B (Fig. 107) along with their epimers as well as 19,20-dinorsesterterpenes, yosgadensenol (Fig. 108) and 13-epi-yosgadensenol (Fig. 109) were isolated from *S. yosgadensis* (Topçu *et al.,* 1996 a,b). From *S. limbata* C.A.Meyer two new dinorsesterterpenes were isolated, 6-dehydroxyyosgadensenol (Fig. 110) and 6-dehydroxy-13-*epi*-yos gadensenol (Fig. 111) (Ulubelen *et al.,* 1996).

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5 ESSENTIAL OILS OF *SALVIA* SPR: EXAMPLES OF INTRASPECIFIC AND SEASONAL VARIATION

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INTRODUCTION

The volatile oils of sage are chemically complex mixtures, often containing in excess of 100 individual components (Waterman 1993, Hay and Waterman 1993), although terpenoid molecules predominate. They have low boiling points and can be recovered from the plant tissues by steam distillation. In addition to flavouring foods, volatile oils can also act as antioxidants and preservatives against food spoilage, while a broad range of applications in aromatherapy and health care has been observed during the last fifteen years (Hay and Waterman 1993).

Volatile oils are synthesized, stored and released to the environment by a variety of epidermal or mesophyll structures, whose morphology tends to be characteristic of the taxonomic group (Hay and Svoboda 1993). The terpene components of the essential oil of sage are synthesized in the cells of the leaf trichomes and stored in the extracellular space within these structures.

In the present chapter we present representative information on the intraspecific and environmental variation of essential oil composition and production in selected *Salvia* species, in addition to data presented by other authors in this volume (e.g. A.Dweck, K.C.Baser, R.Karousou *et al.,* A.Ulubelen, A Jager and J.van Staden). Aspects of essential oil biosynthesis and the analysis of their quality will not be dealt with, since these issues have been extensively reviewed elsewhere (Waterman 1993, Boelens and Boelens 1997).

ESSENTIAL OIL COMPOSITION IN SELECTED *SALVIA* SPECIES

S. aethiopis

The volatile oils of flowering parts of *S. aethiopis* (collected from Spain) were examined by GC-MS. The main, constituents of the essential of *S. aethiopis* were acopaene (10.43–9.15%), germacrene D (10.46–4.95%) and bicyclogermacrene (41.48–29.54%) (Torres *et al.*, 1997).

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S. aytachii

The essential oil, water-distilled from aerial parts of *S. aytachii,* a recently described Turkish endemic, was analyzed by GC-MS (Baser *et al.,* 1997). Fifty-eight compounds were characterized representing 98.41% of the oil. The main components were camphor (30.78%) and 1,8-cineole [eucalyptol] (27.28%).

S. clevelandii

The essential oil, steam-distilled from leaves of flowering plants of *S. clevelandii* (cv. Winifred Gilman), was analyzed by GC-MS. The main constituents were camphor (31.73±5.75%) and 1, 8-cineole [eucalyptol] (20.14±2.4%) (Tucker *et al.,* 1996).

S. euphratica

Baser *et al.* (1998) analyzed the water-distilled essential oil from flowering aerial plants of *S. euphratica* var. *euphratica*. Ninety-seven components representing 89% of the oil were characterized. The major components were trans-pinocarvyl acetate (16.8%) and myrtenyl acetate (14.1%). The essential oil yield was 0.04%.

S. hispanica

Chia *(S. hispanica)* foliage is an excellent source of monoterpene and sessquiterpene essential oils that should be useful as flavours, fragrances and medicinals. Fifty two components were detected by GC analysis in hydrodistilled essential oil from chia leaves (Ting *et al.,* 1997). Most components were sesquiterpenes, mainly ß-caryophyllene, globulol, γ-muurolene, α-humulene, germacrene-B and widrol. The two most prominent monoterpenes were ß-pinene and linalool. The quantitative (but not the qualitative) composition of the essential oil changed during plant development: the percentage composition of ß-caryophyllene, globulol and α-muurolene tended to increase during the growing season as did many of the lesser components. ß-Pinene tended to decrease, whereas α-humulene remained fairly constant.

S. hydrangea

The major constituents of the essential oil of aerial parts of *S. hydrangea* collected from Iran are spathulenol (23.1%), 1–8-cineole (eucalyptol) (12.3%), *a*-pinene (10.04%) and ß-caryophyllene (9.9%) (Rustaiyan *et al.,* 1997).

S. lavandulaefolia

Pecorari (1980) identified ß-pinene (13.8–29.2%), 1, 8-cineole (17.5–24.1%), thujone (2.1–4.2%), camphor (2.8–11.6%) and caryophyllene (4.4–90.4%) as the main constituents of the essential oil of *S. lavandulaefolia,* while Lawrence (1984) detected trace amounts of viridiflorene, ß-spathulene, viridiflorol and humulene epoxide II.

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S. officinalis and *S. fruticosa*

S. officinalis is considered to have the highest essential oil yield among *Salvia* species, along with a higher total ketone content and a lower total alcohol content (Ivanic and Savin 1976, Newall *et al.,* 1996). The major components of the essential oil of *S. officinalis* are *a*- and *ß*-thujones (35–50%, mainly *α*). Others include 1, 8 cineole, borneol, camphor, caryophyllene and linalyl acetate (Newall *et al.,* 1996). Commercial sage may be substituted with *S. fruticosa (S. triloba)* the principal essential oil componet of which is 1, 8-cineole, with *a*-thujone only accounting for 1–5%. Langer *et al.* (1996) analysed by gas liquid chromatography the essential oils of commercially available samples of leaves of *S. officinalis* and *S. fruticosa* (used as medicinal and culinary herbs) obtained by steam distillation and dichloromethane extraction. Although standardized conditions of sample preparation were employed, differences in the composition of the oils were found: steam distillation yielded a reduced amount of the less volatile compounds, and the accuracy of determination was significantly lower than in the case of extraction. The commercial samples, which differed considerably in the composition of their essential oils, were quite different ages of the leaves. Extraction of individual leaves of *S. officinalis* showed a decrease in the *a*-thujone content, with a corresponding increase in the relative amount of camphor, related to leaf age. At Least two chemotypes of *S. officinalis* exist, one with a low a-thujone content (4–8%) and another with a relatively high content (16–32%) (Boelens and Boelens 1997). Owing to the observed variability of the essential oil composition of *S. officinalis,* the relative contents of *a*-thujone, *ß*-thujone and camphor have to be totaled in order to form a significant parameter for the characterization of *Salvia* species. This parameter varied between 45 and 68% in *S. officinalis* and between 4.8 and 15.9% in *S. fruticosa* with a small standard deviation. Consideration of this parameter, together with the amount of 1, 8-cineole [eucalyptol] (*S. officinalis* 2.8–23%, *S. fruticosa* 55–75%), permits the differentiation between these species and respective mixtures.

The terpene alcohols thujol, menthol and thymol were found in *ß*-glucosides in the leaves of Dalmatian *S. officinalis* (Boelens and Boelens 1997).

Kustrak (1988) identified the following constituents in the essential oil (1.55% yield) of *S. officinalis* ssp. *minorf.auriculata:* a-pinene (5.5%), camphene (6.4%), limonene (2.4%), 1, 8- cineole (7.3%), *α*-thujonene (35.3%), *ß*-thujonene (5.6%), camphor (18.1%), linalyl acetate (1.7%), borneol (1.7%) and *α*-terpineol (5.9%). In another subspecies, ssp. *angustifolia,* Pace and Piccaglia (1995) identified 34 components, with the most abundant being *ß*-pinene (7%), 1, 8-cineole (8%), *α*-thujone (39%), *ß*-thujone (3%), camphor (2%), *a*-humulene (12.5%) and globulol (2%).

S. pratensis

The essential oil hydrodistilled from inflorescences of *S. pratensis* subsp. *haematodes* was analysed by Senatore and Feo (1998). The oil content was 0.039% (v/w) on a fresh weight basis. A total of 32 compounds (mainly monoterpenoids) were identified with sabinene (21%) being the main constituent.

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S. sclarea

Souleles and Argyriadou (1997) analyzed the essential oil of *Salvia sclarea* growing wild in Greece. The oil was obtained by steam distillation of the leaves and flowers (yield 2.5%). It has a characteristic odor and refractive index of n^{25}_{D}: 1.535. The preliminary GC analysis showed that the oil contained a large number of components; 72 of the constituents were identified. The identification of the individual GC peaks was made by comparing their retention times with those of the authentic samples and matching the mass spectral (MS) data with those held in a computer library. The oil contained mainly oxygenated monoterpenoids and monoterpenoid hydrocarbons, along with small amounts of oxygenated sesquiterpenoids and sesquiterpenoid hydrocarbons. The major constituents of the essential oil were linalool (17.2%), linalyl acetate (14.3%), geraniol (6.5%), geranyl acetate (7.5%), terpineol (15.1%), nerol (5.5%), neryl acetate (5.2%) and sclareol (5.2%). Other investigators have identified the presence of *a*- and *ß*-pinene, camphene, myrcene, limonene, cis- and trans-ocimeme, p-cymene, terpinolene, cis-3-hexen-1-ol, caryophyllene, terpinen-4-ol, citronellol, *ß*-gurjunene, caryophyllene oxide, germacrene D, (2R, 5E)-2, 12-epoxycaryophyll-5-ene, (2R, 5E)-caryophyl-S-en-12-al, (2S, 5E)- caryophyl-5-en-12-al, isospathulenol, (1R, 5R)-1, 5-epoxysalvial-4(14)-ene and salvial-4(14)-en-1-one (Maurer and Hauser 1983, Boelens and Boelens 1997).

The volatile fraction of the Greek *S. sclarea* differs from many *Salvia* species growing in the Mediterranean region, in regard to compounds, in the variety of its components and their relative quantity. While the oil of *S. sclarea* contains mainly monoterpenoid alcohols such as linalool, geraniol, terpineol, nerol, and linalyl acetate, geranyl acetate, neryl acetate, the essential oils from many *Salvia* species contain *a*- and *ß*-pinenes, camphor, phelladrene, cineol and bornyl acetate as major constituents. *S. aucheri* is very rich in camphor (Holeman *et al.,* 1984), *S. horminum* in pinenes (Kokkalou *et al.,* 1982), *S. lavandulaefolia* has a high content of 1, 8-cineol and camphor (Lawrence *et al.,* 1970), while *ß*-thujone and 1, 8-cineol are the major constituents of the essential oil of *S. officinalis* (Pitarevic *et al.,* 1984), and *S. triloba* (Harvala *et al.,* 1987) (see also above).

Differences and similarities were observed between oils in the Greek and the Yugoslavian *S. sclarea* (Bankovic *et al.,* 1993): a) both oils were characterized by large amounts of linallol and linalyl acetate, b) the oil of the Yugoslavian *S. sclarea* contained lower amount of geraniol (1.2%) from that of the Greek one (8.5%), c) the oil of the Greek *S. sclarea* had remarkably higher quanties of terpineol, manool, sclareol, nerol and neryl acetate which have not been detected in the oil Yugoslavian *S. sclarea*.

The volatile oil of flowering parts of *S. sclarea* collected from Spain contained linalool (32.97%), a-terpineol (5.63%), linalyl acetate (16.85%) and germacrene D (7.57%) as main components (Torres *et al.,* 1997).

In Table 1 the chemical composition of the essential oils of *Salvia sclarea* L., *Salvia lavandulaefolia* Vahl. and *Salvia officinalis* L. is presented, according to data obtained from all cited references.

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Table 1 Chemical composition (%) of the essential oils of *Salvia sclarea* L., *Salvia lavandulaefolia* Vahl. and *Salvia officinalis* L. The presented variation in essential oil composition was calculated from data obtained from all cited references.

Compound	*% in oil S. sclarea*	*% in oil S. lavandulaefolia*	*% in oil S. officinalis*
1. α-Pinene	0.03–0.25	1.93–24.00	0.10–8.70
2. Camphene	0.01–0.10	0.60–14.30	0.80–10.29
3. β-Pinene	0.01–0.30	2.23–48.40	0.20–14.48
4. Mycrene	0.05–2.82	0.50–15.60	–
5. Limonene	0.10–0.77	0.78–58.40	0.56–7.57
6. 1.8-Cineole	0.80–1.30	1.20–54.00	–
7. 2-Hexenal	0.09–0.40	–	–
8. (Z)-β-Ocimene	0.10–1.70	0.20–2.50	0.01–1.00
9. 2-Methyl,2-vinyl,4-isoprenyl-tetrahydrofuran	0.02–0.10	–	–
10. (E)-β-Ocimene	0.06–1.75	0.05–1.30	0.01–1.67
11. Terpinolene	0.10–0.40	0.01–0.80	0.01–0.98
12. Hexenyl Acetate	0.02–0.10	–	–
13. Methyl Heptenone	0.02–0.10	–	–
14. Hexanol	0.10	–	–
15. 3-Hexenol	0.15	–	–
16. Nonanal	0.02–0.10	–	–
17. 2-Hexenol	0.10	–	–
18. Perillene	0.10	–	–
19. cis-Linalool Oxide (Furanoid)	0.01–2.0	–	–
20. α-Ylangene + 1-octen-3-ol	0.10	–	–
21. Nerol Oxide	0.02–0.10	–	–
22. trans-Linalool Oxide (Furanoid)	0.01–1.30	–	–
23. α-Copaene	0.10-3.01	0.01–0.10	0.05–0.45
24. Formic Acid + Benzaldehyde	0.02–0.10	–	–
25. β-Bourbonene	0.08–0.20	–	0.09–0.30
26. β-Cubebene	0.10–0.55	0.05	–
27. Linalool	0.11–31.00	0.10–35.00	0.16–4.70
28. Linalyl Acetate	0.19–74.18	0.01–6.00	0.01–3.50
29. Bornyl Acetate	0.60	0.21–7.70	0.10–6.40
30. β-Caryophyllene	0.40–11.01	0.20–12.80	–
31. Linaly Formate	0.02–0.10	–	–
32. Aromadedrene	0.10	0.10–0.50	0.05
33. Terpinen-4-ol	0.03–0.20	0.10–2.50	0.05–5.10
34. Methyl Carvacrol	0.02–0.10	–	–
35. α-Humulene	0.10–0.25	0.10–6.20	0.18–18.95
36. Neral	0.16–11.30	0.10–0.20	–
37. Lavandulol + β-Farnesene	0.10	–	–
38. Muurolene	0.02–0.10	–	1.34–1.51
39. α-Terpineol	0.20–7.85	0.10–4.00	0.01–3.40
40. Geranyl Formate	1.25	–	–
41. Geranial	0.21–19.40	0.10–0.30	–
42. Neryl Acetate	0.10–5.20	0.10–0.20	–

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Table 1 Continued

Compound	*% in oil S. sclarea*	*% in oil S. lavandulaefolia*	*% in oil S. officinalis*
43. δ-Cadinene	0.12–0.66	0.01–2.80	0.01–1.94
44. Geranyl Acetate	0.30–36.80	0.01–0.70	–
45. Dihydro 8-cumenol	0.02–0.10	–	–
46. Damascenone	0.10	–	–
47. Nerol	0.05–7.40	0.01–0.60	–
48. 8-Cumenol	0.15	–	–
49. Geraniol	0.05–24.50	0.01–0.70	–
50. Caryophyllene Oxide	0.16–2.20	0.10–3.80	0.10–2.17
51. Hydroxy Citronellal	0.02–0.10	–	–
52. Hydroxy Linallol	1.20	–	–
53. Nerolidol	0.02–0.10	–	–
54. 10-epi-γ-Eudesmol	0.02–0.10	–	–
55. Elemol	0.15	–	–
56. Eudesmol	0.02–0.10	–	–
57. Benzyl Tiglate	0.02–0.10	–	–
58. Dodecahydro-3α6,6, 9α-tetramethyl(2,1-β)furan	0.10	–	–
59. Spathulenol	0.01–3.4	–	–
60. Valerianol	0.10	–	–
61. 8,3-epoxy-15,16-Dinozlab-12-ene	0.15	–	–
62. Carvacrol	0.02–0.10	–	–
63. α-Eudesmol	0.20–0.33	–	–
64. β-Eudesmol	0.89–1.50	–	–
65. Thymol	0.02–0.10	–	–
66. Muurolol	0.02–0.10	–	–
67. Endo-8-hydroxy-cycloisolongifolene	0.10	–	–
68. Manoyl Oxide I	0.10	–	–
69. Deutenyl Curcumene	1.25	–	–
70. 13-epi-Manoyl Oxide	0.15	–	–
71. Manool	2.50	1.94–3.57	0.11–5.91
72. Sclareol	0.10–5.20	–	–
73. (Z)-3-Hexenol	0.10–0.29	–	–
74. (E)-2-Hexenol	0.11–0.19	–	–
75. Hexenal	0.03–0.07	–	–
76. (Z,Z)-allo-Ocimene	0.05	–	–
77. Sabinene	0.01–0.06	0.07–5.80	0.01–3.08
78. α-Terpinene	0.01–0.05	0.01–0.80	0.01–0.38
79. α-Phellandrene	0.05	0.01–5.00	0.06–0.37
80. β-Phellandrene	0.07	–	0.10–0.90
81. Camphor	0.68–0.88	1.30–36.10	0.40–44.00
82. Humulene	1.30	0.10–0.50	0.02–16.40
83. p-Cymen-8-ol	0.09	0.10	0.05–0.13
84. Epoxy Linalyl Acetate 1	1.25	–	–
85. Epoxy Linalyl Acetate 2	1.12	–	–

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Table 1 Continued

Compound	*% in oil S. sclarea*	*% in oil S. lavandulaefolia*	*% in oil S. officinalis*
86. Caryophyllene	0.40–3.00	–	0.10–10.00
87. Germacrene D	0.36–48.39	–	–
88. Sclareol Oxide	0.07–0.53	–	–
90. α-Terpilyl Acetate	0.05–5.00	0.10–11.20	0.17–0.90
91. γ-Terpinene	0.40–0.70	0.05–1.30	0.01–1.03
92. p-Cymene	0.01–0.90	0.01–4.60	0.10–2.00
93. cis-Anhydrolinalool	0.01–0.02	–	–
94. trans-Anhydrolinalool	0.01–0.18	–	–
95. 1-octen-3-ol	0.03	–	0.20–0.69
96. cis-α-Bergamotene	0.30	0.10–0.80	–
97. Isoborneol	0.10	0.10–1.90	0.10–2.80
98. trans-Sabinenehydrate	0.20	0.10–1.70	0.10–0.20
99. trans-2,6-dimethyl-2, 7-octadien-2,6-diol	0.48	–	–
100. 3-acetoxy-2,6-dimethyl-3, 7-octadien-2-ol	2.45	–	–
101. 2,6-dimethyl–1,7-octadien-3, 6-diol	0.10	–	–
102. 3-acetoxy-2,6-dimethyl-1, 7-octadien-6-ol	0.25	–	–
103. α-Cubenene	0.12	0.05–1.10	–
104. δ-Elemene	0.39	–	–
105. β-Elemene	0.15	–	–
106. β-Selinene	0.83	–	–
107. Bicyclogermacrene	5.51	0.01–1.50	–
108. (E,E)-α-Farnesene	0.10	–	–
109. T-Cadinol	0.23	–	–
110. Torreyol	0.51	–	–
111. δ-Terpineol	–	0.01–0.70	–
112. Salvene	–	0.06	–
113. Tricyclene	–	0.06–0.50	0.16–0.77
114. α-Thujene	–	0.01–0.70	0.01–1.40
115. cis-Alloocimene	–	0.01–0.50	–
116. α-p-Dimethylstyrene	–	0.30–0.40	0.10
117. α-Pinene Epoxides	–	0.20–0.70	–
118. Borneol	–	0.10–10.00	0.60–15.50
119. Sabinol	–	0.10–2.50	3.40
120. Isobornyl Acetate	–	0.70–4.90	3.39
121. Sabinyl Acetate	–	1.80–6.20	0.30–2.20
122. α-Gurjunene	–	0.20–0.30	0.04–0.14
123. trans-α-Bergamotene	–	0.10	–
124. allo-Aromedendrene	–	0.05–0.40	0.30–0.91
125. β-Bisabolene	–	0.10–0.30	–
126. ar-Curcumene	–	0.10–0.90	–
127. Viridiflorol	–	0.01–10.92	0.35–9.91

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Table 1 Continued

Compound	*% in oil S. sclarea*	*% in oil S. lavandulaefolia*	*% in oil S. officinalis*
128. Carnone	–	0.30	–
129. α-Thujone	–	1.30–22.82	1.20–45.80
130. β-Thujone	–	0.01–4.32	1.02–40.10
131. Terpineol	–	–	0.10–9.10
132. Geranyl Propionate	–	0.42	–
133. cis-Sabinyl Acetate	–	0.01–24.00	–
134. cis-α-Bisabolene	–	0.20	–
135. Isocaryophyllene	–	0.80	–
136. Curcumene	–	0.40	–
137. Humulene Oxide	–	0.20	0.48–0.80
138. cis-Sabinol	–	8.80–19.50	–
139. Caryophyllenol	–	0.10	–
140. δ-Cadinol	–	–	0.08–2.25
141. α-Cadinol	-	–	0.07–0.31
142. Fenchol	–	0.10–0.20	–
143. cis-Sabinenehydrate	–	0.20–0.40	0.10
144. δ-3-Carene	–	0.10	–
145. cis-Salvene	–	–	0.01–0.75
146. Myrcrene	–	–	0.07–3.08
147. para-Cymene	–	–	0.17–0.73
148. β-Cubenene	–	–	0.23
149. trans-Salvene	–	–	0.01–0.12
150. trans-Sabinol	–	–	0.02–7.66
151. Farnesene	–	–	0.50–0.60
152. Carvone	–	–	0.60
153. Estragol	–	–	0.40
154. Fenchone	–	–	0.10–0.20
155. α-Maaliene	–	–	0.05–0.20
156. β-Copaene	–	–	0.05–0.20
157. γ-Cadinene	–	–	0.10
158. Calamenene	–	–	0.10
159. Thujyl Acetate	–	–	0.07–0.19

-: compound never detected in this species.

SEASONAL VARIATION: EFFECTS OF ENVIRONMENTAL FACTORS ON ESSENTIAL OIL PRODUCTION

In order to create an optimal system for growing volatile oil crops it is important to access the crop response to variation in environmental and cultivation management factors. Due to the pronounced xerophytic characteristics of the commercially culti vated sage species, it has been previously assumed that *Salvia* (along with other herbs originating in Mediterranean zones) would produce high-quality oils only under stressful conditions (high temperature, drought, low fertility) (Hay 1993). Lawrence

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(1977) mentioned that Dalmatian sage grown under prairie conditions in Canada possessed a lower volatile oil content than Dalmatian sage, and the oil was of an inferior quality to normal Dalmatian sage oil. However, *Salvia* species display a marked plasticity which allows the adaptation of their phenologies to the growing season in a range of other environments. Furthermore, experiments under controlled conditions have demonstrated that variation in environmental factors such as temperature, irradiance and photoperiod can influence oil yield and quality.

Effect of Light

Bernath *et al.* (1991) cultivated representative sage species (*S. officinalis, S. sclarea, S. aethiopis* and *S. austriaca*) under controlled and field conditions. In growth chambers Hungarian warm (up to 27/17 °C day-night rhythm) and cold (21/11 °C) climatic conditions were simulated using 16 klx and 8 klx light intensities. The changes in plant development and alteration in primary and secondary production were detected. The magnitude and spectra of essential oil accumulation proved to be an identifiable mark of the species. However, the actual level was determined by the environment. A defined modification in the environment can induce a similar change of biosynthesis, accumulation or metabolism of a given component of several species. It has been justified by PCA analysis that by neglecting proper physioecological investigations even separated groups of the same population might consider to be pardoned intraspecific chemotaxa.

In studies carried out by YanLi *et al.* (1996) sage *(Salvia officinalis)* and thyme *(Thymus vulgar is)* plants were grown in a greenhouse under different shade cloths, or without shade, giving light levels of 15%, 27%, 45% and 100% of full sunlight. The total essential oil concentration in sage was highest (0.38% FW) in the plants grown at 45 % of full sunlight, and at this light level, the oil had a higher content of (+)-thujone and a decreased accumulation of camphor as compared with essential oils from sage grown at other light levels. In thyme, the highest essential oil concentration (0.49% FW), and thymol and myrcene contents of the oil, occurred in full sunlight. Leaf length, width, and density of peltate hairs on both herbs decreased with decreasing light intensity.

Effect of the Developmental Stage

It is important to keep in mind that *Salvia* spp. is cropped for leaf oil rather than total plant production, while an inverse relationship exists between oil production and biomass, with the highest yield occuring after flowering is complete (Pitarevic *et al.*, 1984, Putievsky *et al.*, 1986, Hay 1993). For example, Mueller-Riebau *et al.* (1997) indicate that the best harvest time to obtain essential oils from *S. fruticosa* with the highest active ingredients is July. Perry *et al.* (1996) identified the components in essential oils of Dalmatian sage *(Salvia officinalis)* by ^{1}H- and ^{13}C-NMR spectroscopy and by GC-MS. The diterpene manool and several sesquiterpenes were identified for the first time in steam-distilled oil from Dalmatian sage. A rapid GC method was developed and used to analyzed oils from a preliminary study of one

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flowering and four non-flowering accessions of Dalmatian sage, obtained from varied parts of the world and grown in Central Otago, New Zealand. Yields from pilot-scale distillations in summer were significantly higher from non-flowering accessions (0.23% FW) than from flowering accessions (0.14%). Oils from flowering and non-flowering accessions had different compositions, with significantly higher levels of thujones, ß-caryophyllene and viridiflorol determined in flowering accessions. Finally, Yoshida and Sawasaki (1978) found that the maximum clary sage oil content was obtained at the end of the blossom period.

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6 SALVIANOLIC ACIDS AND RELATED COMPOUNDS

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INTRODUCTION

The genus *Salvia* has a variety of more than one hundred species distributed in several regions in China. Thirty of them are used as traditional and folk medicines (Huang 1991). The dried root of *Salvia miltiorrhiza,* called Danshen, is one of the most well known traditional Chinese medicines among these species. It has the effect of promoting blood circulation and removing stasis, and is widely used for the treat ment of coronary heart diseases, cerebrovascular diseases, hepatitis, hepatocirrhosis, chronic renal failure, dysmenorrhea and neurasthenic insomnia. The chemical constituents of *S. miltiorrhiza* have been studied for more than fifty years. But the studies have mainly been focused on the lipophylic diterpenoid quinones. According to traditional Chinese medicinal prescriptions it is used as a decoction. Since the seventies injections of Danshen have been used for the treatment of angina pectoris, myocardial infarction and various types of hepatitis. So there should still be other water soluble active components which are responsible for these biological activities. During our investigation on the biologically active components we isolated various polyphenolic acids from the aqueous extract of *S. miltiorrhiza* and other herbal medicines from this genus. Pharmacological studies of these polyphenolic acids showed potent antioxidant activities (Liu 1992).

CHEMICAL STRUCTURES OF SALVIANOLIC ACIDS AND RELATED COMPOUNDS

Chemical studies on nine *Salvia* species yielded various polyphenolic acids. Eleven of them were depsides of R-(+)-*ß*-(3, 4-dihydroxyphenyl)-lactic acid and a caffeic acid derivative or a caffeic acid dimer forming several types of carbon skeletons (Fig. 1). Except the two known compounds, rosmarinic acid (1.12) and lithospermic acid (1.13), this type of depsides has not been isolated from other plant materials before, so we have given them the names salvianolic acid A (1.1) (Li, 1984), B(1.2), C(1.3), D(1.4), E (1.5) (Ai and Li 1988, 1992), H(1.8), 1(1.9) (Zhang and Li 1993, 1994), J(1.10) (Ai *et al.,* 1994) and isosalvianolic acid C(1.11) (Qian and Li 1992). Salvianolic acid F(1.6) and G(1.7) (Ai and Li 1996, 1991) were two new

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polyphenolic acids, the former was a stilbene derivative, while the latter possessed an unusual tetracyclic dibenzooxepin skeleton.

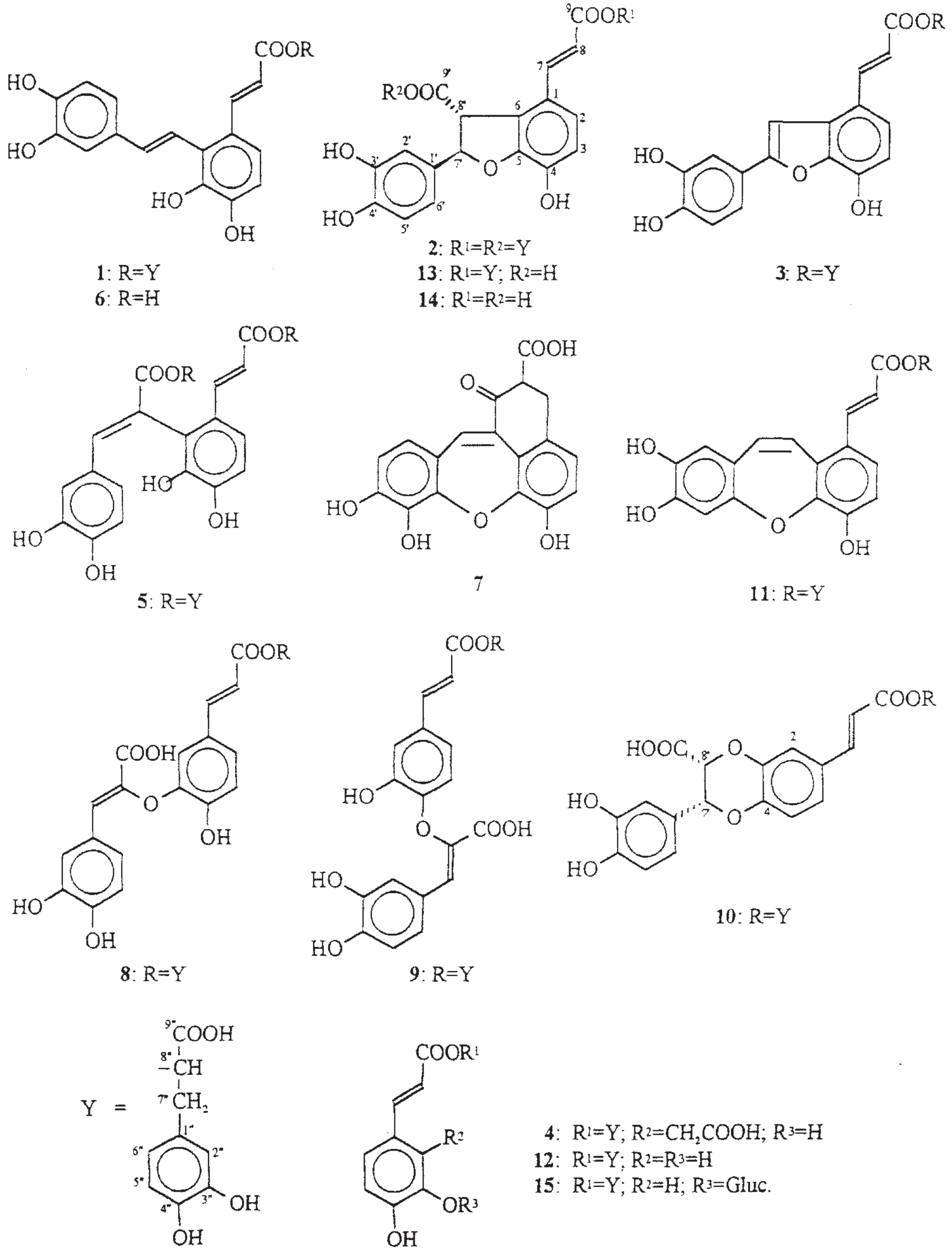

Figure 1 Depsides and polyphenolic acids from *Salvia* species.

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The carbon skeleton of przewalskinic acid A(1.14) (Lu *et al.*, 1991) was similar to that of salvianolic acid B and lithospermic acid. Recently a rosmarinic acid-3-O-ß-D-glucoside named salviaflaside (1.15) (Zhao *et al.*, 1996), and its methyl ester were isolated from the aqueous extract of *S. flava*.

The fact that the carbon skeletons of salvianolic acid B, E, G, H, I, and J were dimers of caffeic acid, suggested that they might be considered as neolignans (Gottlieb 1978). It is noteworthy that this is the first group of neolignans with free phenolic hydroxyls.

EXTRACTION AND ISOLATION OF SALVIANOLIC ACIDS AND RELATED COMPOUNDS

The dried plant material was extracted with H_2O under reflux and the aqueous extract concentrated under reduced pressure. EtOH was added to the concentrate until the EtOH content was 70%. After filtration, the filtrate was concentrated and extracted with $CHCl_3$. The aqueous portion was acidified to pH 3 and succes sively extracted with EtOAc and n-BuOH. Evaporation of the EtOAc and n-BuOH extracts yielded the total phenolic acid fractions which were isolated by the following methods.

SiO_2 Dry Column Chromatography

The total phenolic acid was applied on SiO_2 dry column chromatography with $CHCl_3$-MeOH-HCOOH (85:15:1) as solvent. The column was cut into several equal sections which were individually eluted with warm EtOH. A rough separation according to its polarity was obtained by this method.

Preparative TLC

The sections obtained by SiO_2 dry column chromatography were further isolated by preparative SiO_2-GF_{254} TLC using $CHCl_3$-MeOH-HCOOH (85:15:1) as solvent. The individual flourescent bands were eluted with acetone.

Sephadex LH-20 Column Chromatography

Chromatography over sephadex LH-20 with MeOH as solvent has the advantage of high recovery of the phenolic acids. It can be used for isolation of the total phenolic acid as well as for purification of fractions obtained from the above mentioned methods.

Low Pressure Liquid Chromatography

Minor phenolic acids which were tedious to be isolated were applied on LPLC using Lichoprep RP-18 column and MeOH-H_2O-HCOOH (45:55:1) as solvent. The

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regioisomeric compounds salvianolic acid H and I were successfully isolated by this method.

UV SPECTRA OF SALVIANOLIC ACIDS AND RELATED COMPOUNDS

The UV spectra of salvianolic acids and related compounds exhibited characteristic absorptions for a cinnamoyl chromophore at 230, 290 and 320 nm. A shoulder at 220 nm was observed in the spectra of some compounds. A highly conjugated system in the structure increased the intensity at 310 nm and caused an additional absorption maximum at 340 nm. Salvianolic acid B and lithospermic acid which possess an aryl dihydrobenzofuranoid skeleton revealed absorption at 253 nm.

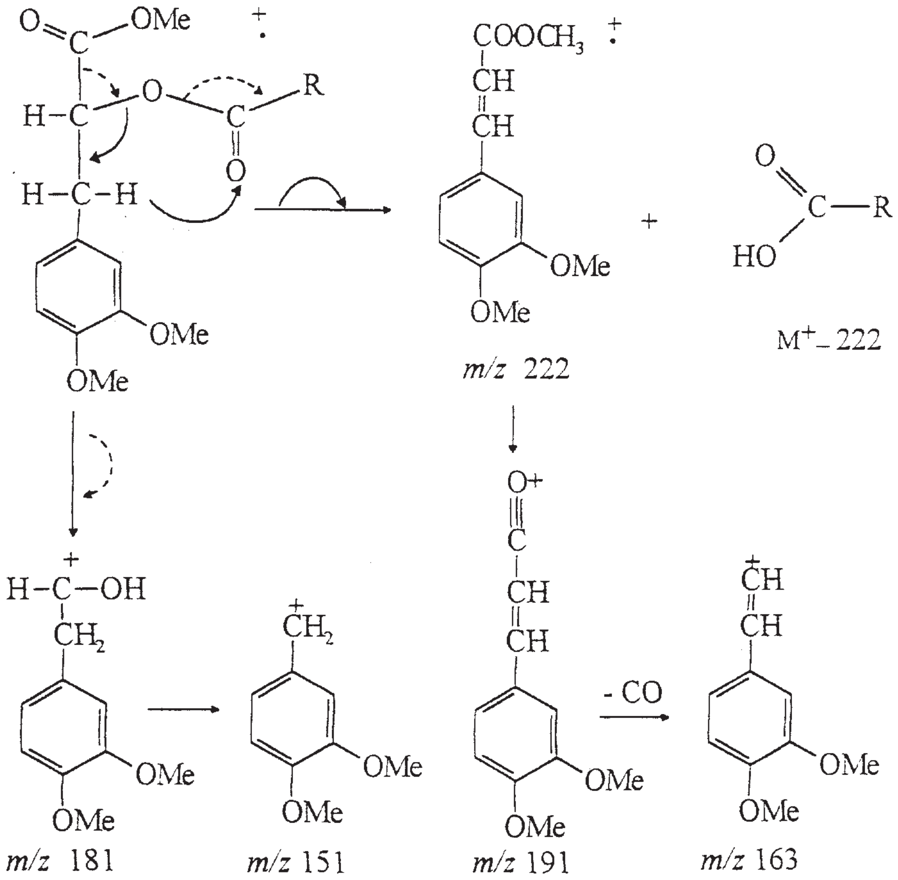

Figure 2 MS fragmentation of methylated salvianolic acids.

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MS OF SALVIANOLIC ACIDS AND RELATED COMPOUNDS

The fact that salvianolic acids are highly polar compounds the molecular ion could only be obtained by FDMS or FAB MS. However the methylated product revealed diagnostic fragmentation pattern of a *ß*-(3, 4-dimethoxyphenyl) lactic ester at *mlz* 222, (M±222), 191, 181, 163, and 151 (Fig. 2).

NMR SPECTRA OF SALVIANOLIC ACIDS AND RELATED COMPOUND

The ′H NMR spectra of salvianolic acids and related compounds revealed signals of *trans* disubstituted double bond at δ 7.60–8.00 (α-H, J=16 Hz) and δ 6.20–6.30 (ß-H, J=16 Hz). The 3, 4-dihydroxyphenyl lactic moiety showed signals of two methylene proton doublets at δ 3.0–3.5, an oxygen bearing methine proton at δ 4.43–4.50 (*dd*, J=4/7Hz) and three aromatic protons at δ 6.70–6.90. A pair of doublets at δ 4.43–4.50 (J=5.4, C-8H) and δ 5.90–6.00 (J=5.4, C-7H) indicated the presence of a *trans* disubstituted dihydrobenzofuranoid skeleton for salvia nolic acid B and lithospermic acid. A relative downfield olefinic proton singlet at δ 8.0±0.1 for salvianolic acid E suggested that the two phenyl groups of the carbo xystilbene should be *cis* oriented. In the *trans* isomer this proton signal was less deshielded and appeared at δ 7.2. The dibenzooxepin skeleton of isosalvianolic acid C was deduced from the fact that the two olefinic signals at δ 6.94 and δ 7.00 possessed a coupling constant of J=11.5 Hz. A pair of oxygen bearing methine proton doublets at δ 4.81 and 5.31 with a coupling

Table 1 Specifie rotation and occurrence of salvianolic acids and related compounds.

Compound	α_D *(EtOH)*	*Plant**
Salvianolic acid A	+41	*Sm, Scs, Sc, Sf, Sb, Sp*
Salvianolic acid B	+92	*Sm, Scs, Sc, Sf, Sch, Sb, Sp, Sy*
Salvianolic acid C	+70	*Sm, Scs, Sc, Sb, Sp*
Salvianolic acid D	+68	*Sm, Sch*
Salvianolic acid E	+59	*Sm*
Salvianolic acid F		*Sm*
Salvianolic acid G	–100	*Sm*
Salvianolic acid H	+63	*Scs*
Salvianolic acid I	+71	*Sc, Scs*
Salvianolic acid J	+26	*Sf*
Isosalvianolic acid C	+39	*Sch, Scs, Sc, Sb*
Rosmarinic acid	+67	*Sm, Scs, Sc, Sf, Sch, Sb, Sp*
Lithospermic acid	+12	*Sm, Scs, Sc, Sch*
Przewalskinic acid		*Sprz*
Salviaflaside	+18.5	*Sf*

Sm: Salvia miltiorrhiza Bunge, *Scs: Salvia cavaleriei* Levl.var. *simplicifolia* Peter- Stibal
Sc: Salvia cavaleriei Levl., *Sf: Salvia flava* Forrest ex Dieles, *Sch: Salvia chinensis* Benth,
Sb: Salvia bowleyana Dunn, *Sp: Salvia prionitis* Hance, *Sy: Salvia yunnanensis* C.H. Wright,
Spr: Salvia przewalskii Maxim

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Table 2 UV spectral data of Salvianolic acids and related compounds.

Compound	*λ max (EtOH) nm (log ϵ)*
Salvianolic acid A	205 (4.27), 218 (4.19), 287 (4.08), 305 (4.05), 335 (4.00)
Salvianolic acid B	203 (4.95), 253 (4.13), 288 (4.16), 308 (4.09), 330 (4.05
Salvianolic acid C	202 (4.79), 215 (sh,4.52), 288 (4.40), 312 (4.32), 330 (4.31)
Salvianolic acid D	203 (4.73), 220 (sh,4.36), 288 (4.14), 310 (4.13)
Salvianolic acid E	205 (4.92), 299 (4.28), 308 (4.32), 330 (4.33)
Salvianolic acid G	203 (5.57), 257 (5.00), 271 (4.98), 280 (4.94), 315 (4.87), 403 (4.76)
Salvianolic acid H	205 (4.76), 220 (sh, 4.49), 286 (4.34), 300 (4.32), 318 (4.38)
Salvianolic acid I	205 (4.76), 220 (sh, 4.50), 256 (4.09), 286 (4.39), 300 (4.35), 318(4.38)
Salvianolic acid J	203 (4.87), 218 (sh, 4.53), 288 (4.24), 323 (4.21)
Isosalvianolic acid C	202 (4.77), 222 (sh, 4.46), 288 (4.21), 326 (4.30), 340 (4.24)
Rosmarinic acid	203 (4.52), 220 (4.48), 290 (4.07), 328 (4.14)
Lithospermic acid	203 (4.52), 220 (4.48), 255 (4.22), 288 (4.22), 310 (4.21)

Table 3.1 Selected ^1H NMR spectral data of salvianolic acids and related compounds.

H	*1a*[1)]	*2*	*2a*	*2b*[2)]	*3*	*3a*	*3b*	*4*	*4a*
5	6.90	6.91	6.90	6.93	6.84	6.81	6.84	6.88	6.90
	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8
6	7.30	7.28	7.20	7.27	6.99	6.95	7.00	7.24	7.40
	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8
7	8.10	7.66	7.61	7.79	7.97	8.00	7.96	7.96	7.88
	J=16	J=16	J=16	J=16	J=16	J=16	J=16	J=16	J=16
8	6.32	6.30	6.21	6.31	6.55	6.52	6.32	6.32	6.32
	J=16	J=16	J=16	J=16	J=16	J=16	J=16	J=16	J=16
2′				6.92	7.41	7.44	7.44		
	6.90	6.80	6.80	J=2	J=2	J=2	J=2		
5′	|	|	|	6.93	7.52	7.42	7.42		
	|	|	|	J=8	J=8	J=8	J=8		
6′	7.10	7.00	6.90	6.84	7.54	7.54	7.56		
				J=2/8	J=2/8	J=2/8	J=2/8		
7′	7.16	5.90	6.05	6.07					
	J=16	J=5.2	J=5.4	J=5.4					
8′	6.64	4.47	4.43	4.51	7.55	7.20	7.40		
	J=16	J=5.2	J=5.4	J=5.4					
OCH_3	3.72		3.72	3.80		3.77	3.97		3.68
	3.80		(×3)	3.82		3.83	4.04		3.76
	3.84		3.88	3.88		3.84	4.12		3.83
	(×2)		(×3)	(×2)		3.96			(×2)
	3.92		3.90	3.96		4.01			3.86
	3.96		(×2)			4.10			3.91
	(×2)		3.98						
2-CH_2								3.90	3.86
solvent*	C	A	C	C	A	C	C	A	C

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Table 3.2 Selected 1H NMR spectral data of salvianolic acids and related compounds.

H	*4b*	*5*	*5a*	*6*	*6a*	*7*	*7a*	*8*	*8a*
2								7.08	6.91
								J=2	J=2
5	7.04	7.01	7.10	6.80	6.88	6.95	6.97	6.81	6.96
	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=8
6	7.51	7.40	7.60	7.20	7.42	6.95	6.95	7.13	7.15
	J=8	J=8	J=8	J=8	J=8	J=8	J=8	J=2/8	J=2/8
7	7.85	7.56	7.65	8.00	8.11	2.31	2.33	7.50	7.53
	J=16	J=16	J=16	J=16	J=16	J=6/15	J=6/15	J=16	J=16
						2.49	2.54		
						J=9/15	J=9/15		
8	6.31	6.28	6.30	6.14	6.31	5.52	5.43	6.20	6.20
	J=16	J=16	J=16	J=16	J=16	J=6/9	J=6/9	J=16	J=16
2′								7.34	7.39
		6.60	6.50	6.90	6.80			J=2	J=2
5′		|	|	|	|	7.02	7.14	6.98	6.83
		|	|	|	|	J=8.3	J=8	J=8	J=8
6′		6.90	6.90	7.10	7.10	7.20	7.38	7.29	7.23
						J=8.3	J=8	J=2/8	J=2/8
7′		7.97	8.10	6.96	7.22	7.73	7.78	7.30	7.41
				J=16	J=16				
8′				6.68	6.69				
				J=16	J=16				
OCH_3	3.80		3.50		3.78		3.47		3.71
	3.90		3.68		3.79		3.84		3.74
			3.73		3.92		3.94		3.78
			3.76		(×2)		(×2)		3.82
			3.84		3.96				3.84
			3.89						3.87
			(×2)						3.98
			3.90						
			(×2)						
			3.99						
2-CH_2	3.86								
solvent*	C	A	C	A	C	A	C	A	A

constant of J=3 Hz indicated the presence of a *cis* disubstituted benzodioxane skeleton for salvianolic acid J. The location of the aryl and carboxyl groups on the dioxane ring was determined by selective DEPT experiments. The calculated chemical shift value of the aryl bearing methine proton (δ 5.31, C-7H) should be at lower field compared to that of the carboxyl bearing methine proton (δ 4.81, C -8H). When the methine proton signal at δ 5.31 and the aromatic proton doublet at δ 7.28 (J=2, C-2H) were selectively irradiated, an enhancement of the C-4 signal at δ 142.9 was observed in both cases. Thus this carbon (C-4) is coupled individually through three bonds to C-7H and C-2H.

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Table 3.3 Selected [1]H NMR spectral data of salvianolic acids and related compounds.

H	*8b*	*9*	*9a*	*10*	*11b*	*12*	*12a*	*13*	*15*
2	7.08 *J*=2	7.18 *J*=2	7.12 *J*=2	7.28 *J*=2		7.21 *J*=2	7.40 *J*=2		7.41 s,br
5	7.13 *J*=8	6.79 *J*=8	6.75 *J*=8	6.88 *J*=8	7.11 *J*=8	6.88 *J*=8	6.84 *J*=8	7.00 *J*=8	6.83 *J*=8.3
6	7.28 *J*=2/8	7.01 *J*=2/8	6.97 *J*=2/8	7.14 *J*=2/8	7.53 *J*=8	7.04 *J*=2/8	7.08 *J*=2/8	7.32 *J*=8	7.18 *J*=8.3
7	7.50 *J*=16	7.60 *J*=16	7.61 *J*=16	7.48 *J*=16	7.80 *J*=16	7.60 *J*=16	7.68 *J*=16	7.72 *J*=16	7.41 *J*=16
8	6.23 *J*=16	6.39 *J*=16	6.33 *J*=16	6.40 *J*=16	6.30 *J*=16	6.32 *J*=16	6.32 *J*=16	6.36 *J*=16	6.33 *J*=16
2′	7.49 *J*=2	7.30 *J*=2	7.37 *J*=2	6.74 *J*=2	6.90			 6.80	
5′	6.92 *J*=8	6.80 *J*=8	6.82 *J*=8	6.61 *J*=8	6.80			| |	
6′	7.28 *J*=2/8	7.12 *J*=2/8	7.19 *J*=2/8	6.68 *J*=2/8				7.00	
7′	7.41	7.37	7.38	5.31 *J*=3	6.94 *J*=11.5			5.90 *J*=5.2	
8′				4.81 *J*=3	7.00 *J*=11.5			4.81 *J*=5.2	
OCH_3	3.68 3.79 3.98		3.74 (×2) 3.77 3.84 3.86 (×2) 3.98		3.72 3.77 3.91		3.75 3.87 (×2) 3.97 (×2)		
Glu-1″									4.79 *J*=7.2
solvent*	A	A	C	D	D	A	C	A	D

*A: $(CD_3)_2CO$, C: $CHCl_3$, D: DMSO-d_6.
a[1]): methylated compound b[2]): hydrolysis product of methylated compound.

CHEMICAL TRANSFORMATION

Methylation of Salvianolic Acids and Related Compounds

Due to the presence of phenolic 3, 4-dihydroxyl groups in the structure, salvianolic acids and related compounds are very labile in alkaline solution. So methylation of this type of compounds is carried out under mild condition. Anhydrous K_2CO_3 (3.3 g) was suspended in a solution of salvianolic acid A (600 mg) in anhydrous acetone (30 ml) under an atmosphere of N_2. Dimethylsulfate (11 ml) was added dropwise, after stirring for 6 h, a second portion of dimethylsulfate (5 ml) was added and stirring was continued

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Table 4 ^{13}C NMR spectral data of salvianolic acids and related compounds.

C	*2a*[1)]	*2b*[1)]	*3b*	*7*	*7a*	*8a*	*9*	*10*	*11b*	*15*
1	128.0	125.1	119.2	127.1	126.7	127.5	130.8	128.0	124.5	125.8
2	132.5	132.5	130.0	127.6	127.0	112.9	116.7	116.7	130.1	116.2
3	148.7	148.6	142.5	139.3	139.6	146.2	146.3	144.0	145.7	145.5
4	149.5	149.5	149.0	144.6	147.1	151.3	148.3	142.9	152.4	149.3
5	111.4	111.4	106.5	140.6	139.1	112.7	116.5	117.1	112.7	116.0
6	116.4	117.4	111.9	120.2	116.3	124.2	122.2	122.5	124.2	124.0
7	142.0	141.1	141.0	40.7	39.2	145.3	146.7	145.0	134.0	144.7
8	121.0	120.5	117.1	37.7	36.9	115.4	116.4	115.5	119.6	116.5
9	165.8	167.3	168.0	173.1	171.2	166.1	168.2	165.9	167.2	166.1
1′	124.7	124.7	122.0	120.1	118.9	125.3	125.8	127.7	121.9	
2′	108.9	109.0	125.0	127.6	132.7	113.0	118.2	114.6	111.6	
3′	149.5	149.5	146.0	140.6	142.2	149.1	146.4	145.3	150.4	
4′	146.2	146.2	150.0	151.9	157.3	150.8	148.8	145.4	146.0	
5′	113.2	113.4	118.0	125.8	123.6	111.3	115.7	115.5	105.3	
6′	117.9	118.0	108.3	129.2	131.4	124.9	124.9	118.2	150.7	
7′	87.3	87.5	157.0	114.4	110.5	128.0	129.2	75.0	131.2	
8′	55.8	55.9	99.7	125.9	124.1	137.8	139.3	76.5	124.4	
9′	170.2	171.8		169.1	167.8	163.9	167.0	169.6		
1″	128.0					128.6	129.5	127.2		129.8
	128.5									
2″	114.4					113.0	117.7	116.8		115.9
	111.4									
3”	148.1					148.4	146.1	145.0		143.4
	148.3									
4”	148.7					149.1	145.2	145.1		144.0
	149.5									
5″	112.7					111.7	116.4	116.8		115.4
	112.7									
6″	121.1					121.6	121.7	120.0		119.8
	121.6									
7″	36.6					37.2	37.9	36.3		37.0
	37.0									
8″	73.1					73.1	74.9	73.5		73.4
	74.1									
9″	169.3					170.3	173.7	171.2		172.4
	170.2									
OCH_3	52.2	51.5			51.5	52.2				101.8[a]
	52.2	51.7				52.4				75.5[b]
	55.8	55.9	55.7		56.0	55.9			55.9	75.8[c]
	(×4)	(×3)								69.9[d]
	56.1		55.5		56.9	55.6			55.7	77.1[e]
	(×2)									60.7[f]
	56.4		55.9		61.3	56.0			56.1	
						(×2)				
						56.4				
solvent*	C	C	D	A	C	A	D	D	D	D

*C:$CHC1_3$, A:$(CD_3)_2CO$, D: DMSO-d6 [a, b, c, d, e, f]: Glu-1″, 2″, 3″, 4″, S″, 6″.
a[1)] : methylated compound b[2)] : hydrolysis product of methylated compound.

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for another 6 h. The mixture was filtered and evaporated under reduced pressure. The residue was applied on a SiO_2 (40 g) column and consecutively eluted with hexane, hexane -$CHCl_3$, $CHCl_3$ and $CHCl_3$-MeOH. Fractions of $CHCl_3$-MeOH (9:1–7:3) showed a yellowish green flourescent spot on TLC. After removal of the solvent, the residue was purified by preparative TLC with $CHCl_3$-MeOH (99:1) as solvent, yielding 460 mg of methyl hexamethyl-salvianolate A.

Hydrolysis of Methylated Salvianolic Acids

Hydrolysis of methylated salvianolic acids in 10%KOH/MeOH under reflux yielded the optically active ß-(3, 4-dimethoxyphenyl)-lactic acid and the methylated caffeic acid derivative or dimer. For alkaline sensitive compounds, such as salvianolic acid B, sodium methoxide under mild condition was used for the hydrolysis of the methylated compound. A base-induced racemation of methyl-ß-(3, 4-dimethoxyphenyl)-lactate occurred under this condition. To a cool solution of dimethyl heptamethylsalvianolate B (1 g) in $CHCl_3$ (50 ml), 5 ml of 0.6N MeONa was added dropwise, and the mixture was stirred at 0° for 1 h. After acidification and work-up with EtOAc and H_2O, the organic layer was concentrated and the residue applied on preparative TLC using C_6H_6-EtOAc-HCOOH (80:20:1) as solvent. Elution of the individual bands with acetone yielded 335 mg of the methylated aryl-dihydrobenzofuranoid compound and 250 mg of (R, S)-methyl-ß-(3, 4-dimethyoxyphenyl)-lactate.

Transformation of Salvianolic Acid A

Oxidative cyclization of salvianolic acid A in the presence of trace amount of acid yielded salvianolic acid C. A 20×20 cm TLC plate was impregnated with $CHCl_3$-MeOH-HCOOH (85:15:2). After evaporation of the solvent 50 mg of salvianolic acid A was applied and the plate left for 48 h at room temperature. Multiple devel opment (×3) with $CHCl_3$-MeOH-HCOOH (85:15:1) yielded a dark yellow fluorescent band and a bright green fluorescent band. Elution of the individual bands with acetone yielded 10 mg of salvianolic acid A and 20 mg of salvianolic acid C.

Transformation of Salvianolic Acid B

Base-induced ring opening of the dihydrobenzofuran skeleton of salvianolic acid B led to the formation of the carboxystilbene skeleton of salvianolic acid E. Anhydrous K_2CO_3 (50 mg) was suspended in a solution of salvianolic acid B (70 mg) in anhydrous acetone (5 ml). Dimethylsulfate (0.5 ml) was added and the mixture refluxed under an atmosphere of N_2 for 10 h. Usual work-up yielded 25 mg of dimethylheptamethylsalvianolate B and 40 mg of dimethyl octamethylsalvianolate E.

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IV. CULTIVATION AND BREEDING
7. THE CULTIVATION OF SAGE

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INTRODUCTION

Sage species (*Salvia* spp.) have been used extensively as pharmaceutical herbs since antiquity. From ethnobotanical surveys (Palevitch *et al.,* 1982; Skoula, 1994) it follows that infusions from dry or fresh plant parts are used against abdominal pains, coughs, throat-aches, stomatitis, gingivitis, tooth-aches, diarrhoea, stomac-aches, diabetes, hypertension, rheumatism and skin diseases. They are also used as abortifacients, expectorants, cerebral sedatives, psychotropics, stomach stimulants, for hair care, cleaning of crockery etc. Most of the product is still collected from natural stands, especially in some countries of the Eastern Mediterranean (former Yugoslavia, Albania, Greece, Turkey), whereas systematic cultivation is carried out in Italy, the U.K. and the U.S.A. In view of the globally increasing demand for natural products and, in particular, of the increasing trends in market demands of the main importers (Kaldis *et al.,* 1993), sage cultivation may be a promising alternative to collection. Furthermore, it will provide a means of diversification in agriculture, with a potential for exploitation of marginal lands in the Mediterranean region.

Of the many existing *Salvia* species, those of the main economic importance are the Greek sage (*Salvia fruticosa* Miller=*Salvia triloba* L.), the Dalmatian, garden or common sage (*Salvia officinalis* L.), the apple sage (*Salvia pomifera* ssp. *pomifera* L.=*Salvia calycina* Sibth. & Sm.), and the clary or muscatel sage (*Salvia sclarea* L.). In all species, the essential oils are located in the glandular hairs of all aerial parts with an average concentration between 1.3–3.6% on a dry weight basis. The concentration is maximum in leaves, intermediate in flowers and minimal in stems. The chemical composition of the essential oil varies among species, seasons, and habitats, a fact that leads to significant qualitative differentiations.

In this chapter, aspects of cultivation will be given for all four sage species men tioned above. Although cultivating practices are similar in most cases, possible differences among species will be mentioned when required.

PLANT CHARACTERISTICS

Sage species are perennial shrubs. Stems are long, angular and erect reaching heights between 50 to 100 cm, depending on species and environmental conditions. A

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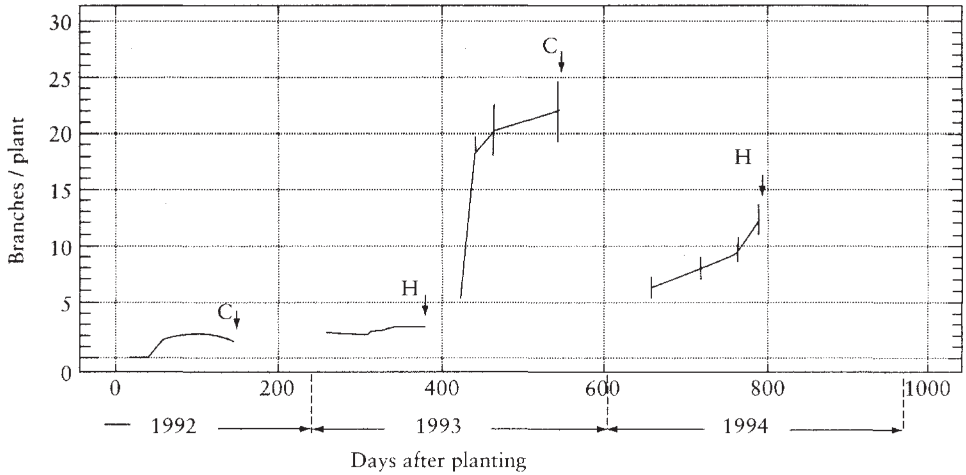

Figure 1 The number of branches produced by sage plants *(S. fruticosa)* cultivated in the field for three years. The timings of harvest in the summer (H) and autumn (C) are indicated (Karamanos 1995).

number of branches (usually 3 to 5) are produced from lateral buds of the main stem. Branching is more intense after cutting (Fig. 1). Leaves are opposite, simple, ovate, and petiolate. The inflorescence is a terminal verticillaster consisting of 4 to 10 violet, blue, lilac or pale-blue flowers. All above ground parts are covered by glandular hairs

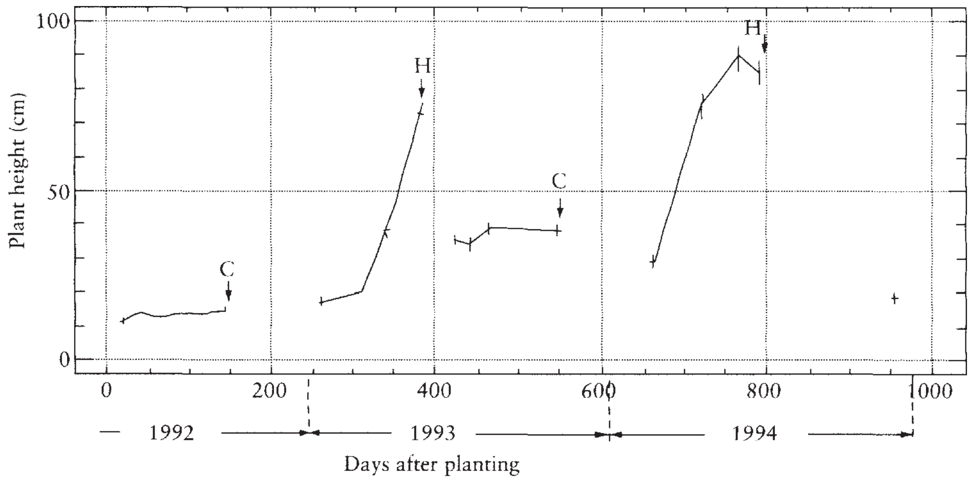

Figure 2 The growth in height of field-grown sage plants *(S. fruticosa)* during cultivation for three years. The timings of harvest in the summer (H) and autumn (C) are indicated (Karamanos 1995).

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which impart a silver colour to mature plants. Flowering starts from mid March to June, depending on climatic conditions, and lasts for about one month.

Plants tend to regrow easily after cutting, both in height and branching, especially after the second year from planting (Figs 1 and 2). There is evidence that sage crops can be productive for more than 10 years when grown in a suitable environment and under the appropriate husbandry (Scroumbis, 1988).

ADAPTATION

All four species originate from Southern Europe. *S. officinalis* grows along the northern part of the Mediterranean (from Spain to the Balkans), whereas *S. fruticosa* and *S. pomifera* are endemic in the Central and Eastern Mediterranean. *S. sclarea* grows within a broader region extending from Spain to Southern Russia (Tutin *et al.,* 1972).

All species can grow from sea level up to altitudes of 1500 m. Consequently, their favourable habitats are found in most subdivisions of the Mediterranean climate (Papadakis, 1975), whereas considerable frost damage occurs in more acute climatic conditions (Rey, 1991). Both herbage and oil yields are reduced in cold and shady environments (Bernath *et al.,* 1991) which induce a reduction in plant size and the density of peltate hairs (Li Yan Li *et al.,* 1996). In general, essential oil concentration tends to be higher in warmer and drier regions (Kargiolaki *et al.,* 1994). Oil chemical composition was also found to depend on environmental conditions (Bernath *et al.,* 1991).

Apart from extremely coarse or fine-textured soils, sage species can be grown over a wide range of soil types, especially in medium-textured, well-drained calcareous soils with a pH around 6.5.

PLANT PROPAGATION

Sage species can be propagated both sexually and asexually.

Sexual Propagation

Seed production from sage plants is abundant. Seeds are spherical, of a fair size (1000 seed weight between 6 to 7g) in comparison with the other Lamiaceae species.

Optimal temperatures for seed germination lie between 10 and 20 ° C for *S. pomifera* and *S. fruticosa*. In the latter species a high germinability was also observed in 25°C (Fig. 3). Seeds of *S. officinalis* also germinate satisfactorily within the range of 10 to 25 °C, whereas those of *S. sclarea* have a broader range of optimal temperatures (10 to 30 °C) (Côme, 1993).

Seed germination is favoured by darkness. There seems to be a slight enhancement by light only in *S. pomifera* (Thanos & Doussi, 1995). On the other hand, germination of *S. fruticosa* seeds is inhibited by blue and far-red lights *(ibid.)*.

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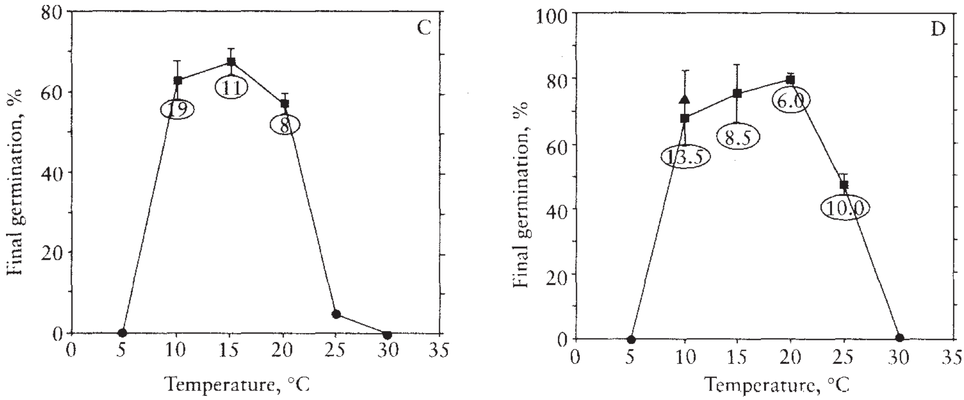

Figure 3 Final seed germination in darkness at constant temperatures for S. *pomifera* ssp. *pomifera* (C) and *S. fruticosa* (D). Circled numbers are T_{50} values in days. Vertical bars represent standard error values (adapted from Thanos & Doussi 1995).

Further research on seed physiology has revealed that sage seeds are dormant to a considerable extent at harvest. This dormancy can be broken by means of either dry storage at 20 °C for 1.5 month or cold treatment at 5 °C for one week. Gibberellic

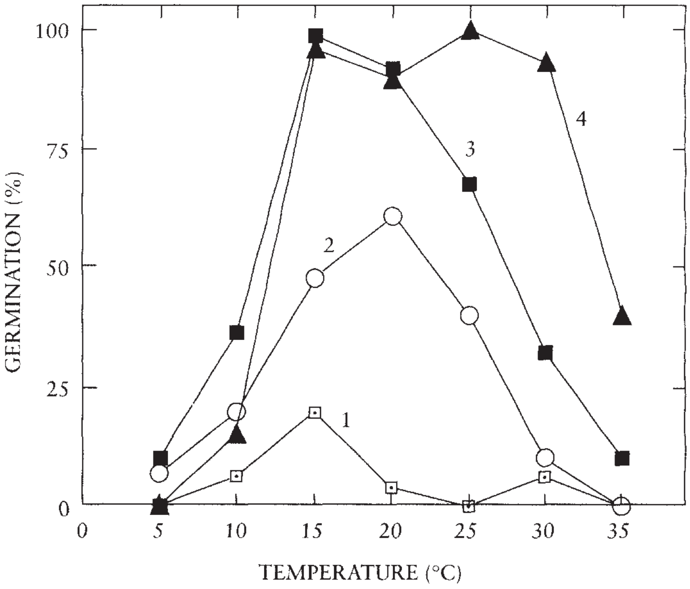

Figure 4 Influence of temperature on final germination obtained after 2 weeks for *S. officinalis* seeds. 1, freshly harvested seeds; 2, seeds stored for 1.5 month at 20° C; 3, seed pretreated for 5 weeks at 5 °C in water; 4, seeds treated with 10^{-3} M GA_3 (Côme 1993).

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acid at a concentration of 10^{-3}M also strongly improves germination of dormant seeds of *S. officinalis* (Fig. 4).

Seed germination is adversely influenced by osmotic stress as well as by oxygen concentrations as low as 5%, i.e. at levels unusually low in nature (Côme, 1994). Storage in air-tight bags for as long as 6 years at 5 ° C does not affect seed germinability; higher temperatures (10 to 30 °C), however, shorten substantially safe storage duration to 2 years only (Kretschmer, 1989). Finally, low seed viability can also be induced by pest and disease attacks.

Seeds are either drilled directly in the field or hand-sown in nurseries. In the former case seed rate varies between 3 to 5 kg/ha (Scroumbis, 1988). In the nursery, the rate is 8 to 10 g/m^2. 70 to 80 m^2 of nursery are required for one hectare of a sage crop *(ibid.)*. Nurseries are set up either in August or in March depending on the scheduled time of transplanting (winter or spring respectively). Seedling care in nursery includes regular waterings, fertilizer application, and weedings. No differences in both herbage and essential oil yields were detected between direct-drilled and transplanted *S. officinalis* crops established in the normal periods (Rey, 1995).

Asexual Propagation

Sage plants are asexually propagated by means of cuttings, secondary stems, and tissue culture. Since tissue culture is the subject of a separate chapter, only the first two techniques are discussed here.

Cuttings

They are segments of 8 to 12 cm length cut from the annual stems and then planted for rooting in pots or trays containing mixtures of soil, perlite and manure. Sage cut tings do not produce roots easily when compared with other Lamiaceae species (Table 1).

Table 1 The rooting percentage and duration for cuttings of three Lamiaceae species (Karamanos 1993).

Species	*Total no of cuttings*	*% rooting*	*Time required (days)*
Origanum hirtum ssp. *hirtum*	306	100	18
Salvia fruticosa	407	53	70
Satureja thymbra	286	81	60

Table 2 The rooting percentage and duration for cuttings of *Salvia fruticosa* treated with different concentrations of indolylbutyric acid (IBA) (Kargiolaki *et al.* 1993).

IBA-conc. (ppm)	*Total no of cuttings*	*% rooting*	*Time required (days)*
500	917	38	53
1000	45	42	47
2000	45	64	47

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Rooting can be promoted by dipping the cuttings in indolylbutyric acid solutions for 5 sec. The usual concentrations are 500 ppm, although higher concentrations have proved to be more effective (Table 2).

Secondary stems

Root-bearing secondary stems can be detached from more than one year-old mother plants. This can be done by carefully excavating around the selected plants. The material obtained can then be successfully transplanted in the field.

LAND PREPARATION

The main aim of land preparation in transplanted crops is weed control. Soil surface is not necessary to be finely ground. Accordingly, one or two ploughings at a depth of 20 to 25 cm followed by one harrowing are usually enough. In the case of drilled crops, however, more than one surface cultivations (harrowing/rotavating) are necessary in order to make the seedbed fine and friable.

If the field is heavily infested by noxious perennial weeds (bermuda grass, Johnsongrass, and yellow nutsedge), a deep ploughing in the summer preceding plant estab lishment is recommended. In this way, the underground propagating organs of the weeds (rhizomes, bulbs) are excavated and desiccated by summer drought.

PLANT ESTABLISHMENT

Planting is carried out in autumn (October-November) or early spring (end of February-March). Autumn planting is more preferable, since a better plant establishment is promoted, except in areas with very cold winters. The choice of the most appropriate plant spacing strongly depends on local conditions (soil fertility, climatic conditions, weeds) and varies from 50 to 60×25 to 40 cm. Denser crops (35×15 cm) have also been successful (Putievski *et al.,* 1986a). On the other hand, wider spacings have been reported (100 cm-apart rows) for seed propagating crops (Macchia *et al.*, 1988). Denser stands reduce the numbers of primary and secondary stems as well as plant dry matter production *(ibid.)*.

Plants are transplanted either manually or using transplanting machines. Grain drills are used whenever seeds are sown *in situ*. In this case, seed density within rows is high, and, therefore, thinning of the seedlings is necessary after plant emergence.

INORGANIC NUTRITION/FERTILIZER APPLICATION

The supply of inorganic nutrients is known to affect plant growth and development. Experiments with sage plants *(S. fruticosa)* grown in solution culture (Economakis, 1993) have shown that nitrogen concentrations of 100 to 150 ppm were high enough to maintain vegetative growth at satisfactory levels. On the other hand, higher

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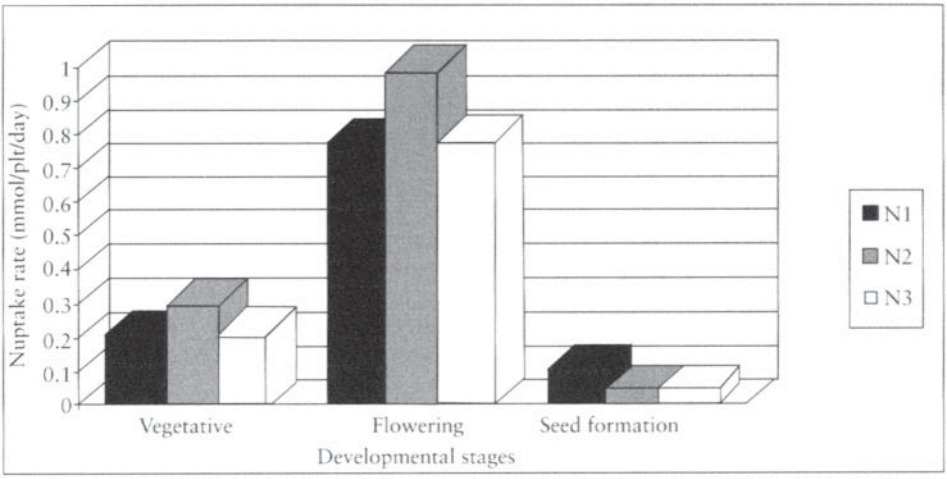

Figure 5 The effect of nitrogen concentration on the nitrogen uptake rate by S. *fruticosa* grown in soilless culture at various developmental stages. N_1: 99 ppm, N_2: 145 ppm, N_3: 184 ppm (Economakis 1993).

concentrations (200 ppm) reduced stem, leaf, and root fresh and dry weights. In the same experiments it was found that the peak demand for nitrogen was observed during flowering (Fig. 5).

In field experiments with four levels of N-fertilization (0, 80, 160, and 240 kgN/ha) on a *S. fruticosa* crop (Karamanos, 1995) it was found that nitrogen application promoted plant height, branching, and herbage yields in comparison with the unfertilized plots. However, no significant difference among the three rates of N-application was detected, a fact indicating that rates of 80 kgN/ha were enough to maintain a satisfactory crop growth. No significant effect of nitrogen on essential oil concentration was observed in this experiment. Similar results were also observed in other experi ments in the field (Bezzi, 1987; Marzi, 1987) and in solution culture (Economakis, 1993). No fertilizer effects were also detected on essential oil composition (Piccaglia & Marotti, 1993). However, essential oil yields increased significantly with N-application mainly because of the observed promotion by nitrogen in herbage yield (Karamanos, 1995).

Vegetative growth was also promoted by phosphorus in solution culture up to a concentration of 34 ppm. Higher concentrations caused a suppresion in growth and development. The peak in phosphorus demand was observed later than that of nitrogen, namely at the seed formation stage (Economakis, 1995). No phosphorus effect on essential oil concentration was detected (Fig. 6).

Experiments concerning iron supply to *S. fruticosa* seedlings have shown that by doubling normal iron concentration in half-strength Hoagland solution an increased gland density in the early stages of growth was observed (Thanos, 1994). The estimated increase in essential oil concentration (ca. 20%) urges for further research on the effects of iron nutrition on sage.

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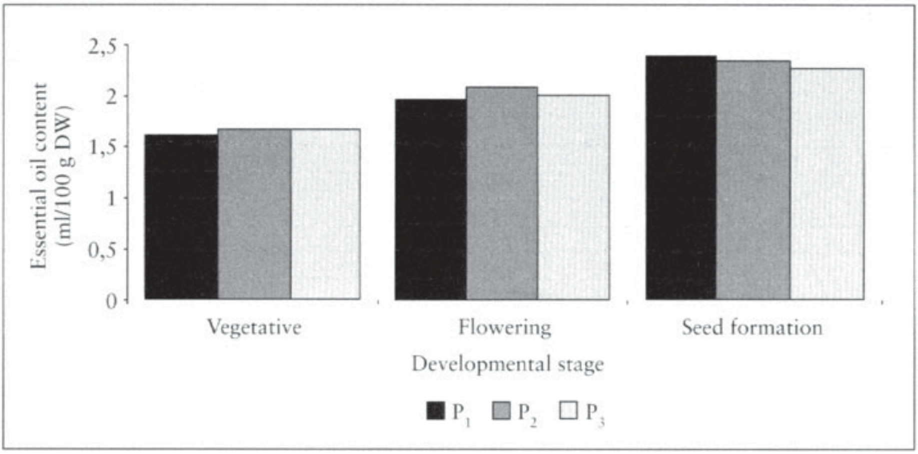

Figure 6 The essential oil concentration of leaves of *S. fruticosa* grown in soilless culture at different levels of phosphorus and various developmental stages. P_1: 17 ppm, P_2: 34 ppm, P_3: 68 ppm (Economakis 1995).

The presence of mycorrhizal hyphae from vesicular arbuscular fungi (VAM) in the roots of *S. pomifera* and *S. fruticosa* (Thanos, unpubl.) implies that the uptake of nutrients (particularly phosphorus) and water can be facilitated to a considerable extent even in dry soils of low fertility.

In practice, fertilizers have to be applied before planting (basic application) to help a quick and efficient crop establishment as well as during growth (surface application) in order to meet the seasonal crop demands. The recommended rates for the basic application depend on the levels of the available macronutrients in soil: 40 to 100 kg N (as ammonium sulfate), 30 to 80 kg P_2O_5, and 30 to 100 kg K_2O per hectare. Fertilizers are mechanically incorporated into the soil. Surface application must be carried out in early spring, 10–15 days before flowering when the maximum uptake rates are observed. Nitrogenous fertilizers are used (usually ammonium nitrate) at rates of 40 to 80 kg N/ha. Incorporation can be done either mechanically between the rows or by means of a light irrigation. Surface application of phosphorus and potassium fertilizers can be carried out in autumn, following the autumn harvest of the crop, whenever the levels of soil available phosphorus and potassium are low. In these cases, the rates vary between 30 to 50 kg of P_2O_5 and 30 to 70 kg of K_2O per hectare. Nitrogenous fertilizers have to be avoided in this case, because of the high risks of leaching by winter rains as well as of the development of winter weeds.

IRRIGATION

The effects of water supply are undoubtedly positive on herbage production of most plants. They are less clear, however, on the concentration of active substances in aromatic and medicinal plants. There are indications that the effects of water may vary substantially among Lamiaceae species of contrasting origin. Thus, one could

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expect a different response of hydrophytes (e.g. *Mentha* species) from xerophytes (e.g. *Lavandula* species) (Bernath, 1986). On this account, sage species, as xerophytes, might exhibit a reduction in their essential oil concentration with increased water supply as a result of the reduction in the concentration of glandular hairs per unit leaf area due to a more intensive cell enlargement. On the other hand, the overall yield in essential oil may increase due to the impressive positive response of herbage growth to water supply. In any case, experimental data concerning the responses of sage species to irrigation are scarce and contrasting.

It is recommended that sage crops can be irrigated in spring to induce a rapid plant growth. Spring irrigation could also be used for incorporating surface fertil izers. A second irrigation after the first cutting considerably helps regrowth and makes possible a second cut in autumn (Marzi, 1987). More than two irrigations are usually not necessary, although irrigations at weekly intervals from spring to autumn have also been reported (Putievsky *et al.,* 1986).

WEED CONTROL

Weed competition is among the major problems in sage cultivation. The periods of marginal crop cover, e.g. the period after planting and those following harvests, are critical in terms of weed control. Hand-weeding is the most labour requiring though the most effective mean of control (Mitchell *et al.,* 1995). Mechanical weeding can be expensive in weedy fields because of the numerous treatments required. Thus, the use of herbicides, exclusively or in combination with mechanical weeding, may provide a solution to the problem of weed control.

A number of chemicals have been tested as herbicides in sage crops. From the preemergence herbicides, chlorbromuron and metobromuron were the best among 40 herbicides tested in field trials in Germany (Pank *et al.,* 1981). Chlorbromuron at a rate of 1 kg/ha, chloroxuron (4 kg/ha), linuron (1 kg/ha), and chloridazon (4.8 kg/ha) were found effective and non-toxic to sage species (*S. officinalis* and *S. sclarea*) in another experiment in New Zealand (James *et al.,* 1991). Chloridazon was also very effective against some dicotyledonous-weeds applied either pre- or postemergence (2.15 and 2.58 kg/ha respectively) (Bouverat-Bernier & Gallotte, 1989). No phytotoxicity of this substance was detected even at higher application rates (4.8 kg/ha) (James *et al.,* 1991). Trifluralin (1 kg/ha), applied before sowing, gave also satisfactory results with no visible phytotoxic effects (Hartley, 1993). Nitrofen or nitrofen plus simazine applied post-emergence at the 6-leaf stage (Pank *et al.,* 1981) and pendimethalin and chlorthal-dimethyl applied in the week following transplanting and before weed emergence (Mitchell *et al.,* 1995) gave also satisfactory results with no visible phytotoxicity. No phytotoxic symptoms were detected from applications of oxyfluorfen against *Oxalis* species (Karamanos unpubl.).

Attention was given on possible effects of the applied herbicides on quality characteristics. Pank (1990), analyzing results from numerous field experiments (22 chemicals on 16 species of aromatic and medicinal plants), found that herbicides with no visible phytotoxic effects on *S. officinalis* tended to increase

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plant leafiness, to reduce dry matter content and increase the thujone content of the essential oil.

In conclusion, it is possible to use herbicides effectively, especially in the first year of plant establishment, depending on the local weed flora. In view of the relatively narrow range of the affected weeds, a combination of chemical with mechanical weed control is recommended in most cases in order to avoid weed competition.

APPLICATION OF PLANT GROWTH REGULATORS

It is possible to modify plant growth to our benefit by exogenously applying plant growth regulators or related substances. El-Keltawi & Croteau (1986a & b, 1987a & b) have examined the effects of a number of growth retardants on sage *(S. officinalis)* yield and quality. Foliar application of phosphon D (chlorphonium) at a rate of 50–100 ppm stimulated plant growth and increased essential oil yield by 50–70%. Ethephon at 250 ppm had similar but less impressive effects. Growth promoters (cytokinins), such as kinetin, diphenylurea, benzyladenine and zeatin at 1–10 ppm induced both a growth promoting effect and a two-fold increase in essential oil yields on a fresh weight basis. An increase in essential oil yield followed by a moderate stunting of growth was caused by the growth regulators AMO-1618 (hydroxy carvacryl trimethylammonium chloride) and DCPTA (2-(3,4-dichlorophenoxy) triethylamine). On the other hand, sage growth and essential oil yield were reduced by daminozide (1000 ppm), whereas chlormequat (250–500 ppm) retarded growth with little effect on oil yield.

The same researchers *(ibid.)* detected significant deviations from normal in essential oil composition of the treated plants: a decrease in camphor and increase in *ß*-pinene by ethephon and daminozide; an increase in thujone and isothujone and a decrease in *ß*-pinene and camphor by phosphon-D; an increase in *ß*-pinene and decrease in isothujone by chlormequat. They ascribed these changes to direct effects of the applied substances on the activities of the relevant biosynthetic enzymes rather than to the induced changes in plant growth and development.

These results, obtained under controlled conditions, clearly show that there is a real perspective of using growth regulators in sage cultivation, provided that adequate information is obtained from field experiments.

HARVEST

The timing of harvest strongly depends on the expected quantity and quality of the product. Fresh matter yield is highest in spring while dry matter yield is highest in summer (Putievski *et al.*, 1986b). Essential oil concentration is lowest in spring (0.7–2.2%), highest in early autumn (2.0-3.4%), and intermediate in winter (1.7–2.5%) (Putievski *et al.*, 1986a; see also Fig. 7). Observations on *S. fruticosa* populations from Crete have revealed that there also exists seasonal variation in the main components of sage essential oil (Fig. 7). The sum of monoterpenes showed their peak value in spring, whereas the sum of thujones (especially α-thujone) and camphor

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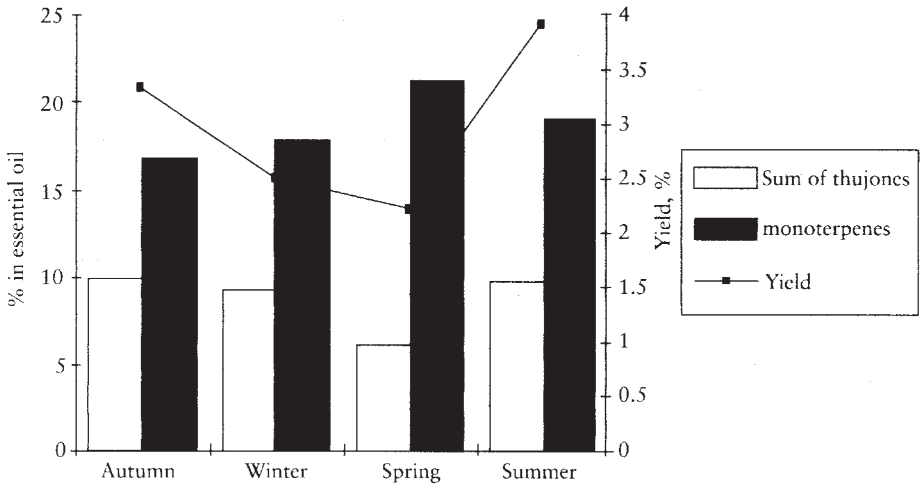

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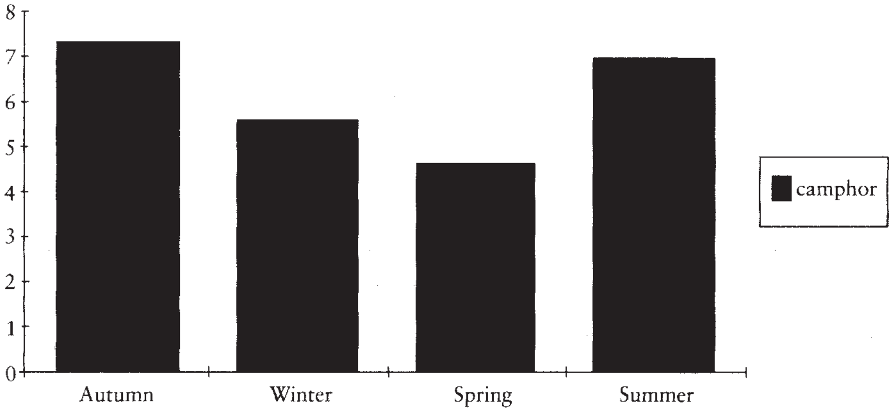

Figure 7 The seasonal variations: (A) in essential oil yield of S. *fruticosa a*s compared with the sums of thujones and monoterpenes. (B) In camphor percentage in the essential oil of *S. fruticosa (Karg*iolaki et al. 1994).

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were lowest in spring and highest in autumn-summer. 1, 8-cineole, the main component of the essential oil, did not exhibit a seasonal variation. Similar results were quoted in Sardinia by Grella & Picci (1988) and in Dalmatia (Pitarevic *et al.,* 1984). Consequently, essential oil quality also differs with harvesting time. According to Putievski *et al.* (1986a), the best quality crop of *S. officinalis* (silver leaf colour, higher than 1.5% essential oil concentration, high in thujones and low in camphor) was obtained in Israel by harvesting in July, and again in October-November. In Greece, the first harvest starts from May in mild climates and ends in July in hilly and mountainous areas (Scroumbis, 1988). In any case, the period start ing from full bloom and ending to seed-set is the more suitable for the first cut for all sage species.

The number of harvests/year depends on the age of the crop. First year crops are harvested only once, in the summer. From the second year onwards crops are har vested usually twice a year (June-July and October-November). More than two cuts per year may be feasible in very mild climates and irrigated crops. In this case, the first harvest takes place in spring (May), the second in middle summer (July), and the third late in autumn (October-November). It has to be noted, however, that successive cuts replenish plant reserves and may restrict the duration of the crop. In addition, total annual yield does not increase significantly from 2 to 4 harvests (Putievski *et al.,* 1986a). For *S. sclarea* it is possible to have a second cut in September/October. This happens in warm climates where blooming stems reappear in autumn. However, this harvest is inferior to the first one in terms both of oil quantity and quality (Scroumbis, 1988).

In *S. fruticosa, S. officinalis,* and *S. pomitera* crops whole plants are cut to a height of 10 to 15 cm from ground surface using mowers or other motorized cutters. Cutting to a height of 5 cm is less successful (Rey, 1991) by restricting regrowth. For *S. sclarea,* however, only the flowering stem is harvested, and, therefore, the machines are adjusted to cut at a higher level.

Herbage yields vary from 3 to 12 tn/ha of dry matter, depending on crop density, soil fertility and water availability (Putievski *et al.,* 1986a & b; Aiello & Clementell, 1987; Bezzi, 1987). Yields from the first year crop are significantly lower (less than half in some cases: Karamanos, 1995) than those from the second year onwards. Leaves are the main yield components (more than 50% in total DW) followed by stems, and flowers (34 and 14% respectively; *ibid.).* Essential oil yields also vary between 110 and 200 kg/ha. Increased leafiness brings about higher oil yields, in view of the highest oil concentration observed in leaves.

POSTHARVEST TREATMENT

The harvested biomass is air-dried under shade. Exposure to direct sunlight has to be avoided in order to prevent evaporation of the volatile compounds of the essential oil. Artificial drying of the essential oil can also be applied at 40 °C for 48 h. Whole plants or plant parts can be used for infusions. Leaves can be mechanically separated from the stems using special equipment, if required, bearing in mind that leaf oil is richer in 1, 8-cineole and myrcene and poorer in *a*-pinene and camphor than that of stems and inflorescences (Putievski *et al.,* 1986b).

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In the case of extraction for essential oil, only fresh material (such as flowering stems in *S. sclarea*) has to be used.

Storage of the dried plant material in darkness for about two years was found to reduce essential oil concentration by 15 to 25% (Paillard, 1994b). Low temperatures during storage (-2 and -18°C) do not offer any advantage in comparison with normal temperatures (20 °C) *(ibid.)*.

It is possible to evaluate a possible qualitative degradation of the essential oil during storage by investigating changes in specific oil components, a-terpinene, a monoterpene, is oxidized or photooxidized to p-cymene (Nitz *et al.*, 1989), and, therefore, may be a good quality indicator. A decrease in α-terpinene by 15 to 30% was observed during storage in dried sage material (Paillard, 1994a). Nevertheless, this decrease may not be important for organoleptic properties, in view of the low percentage of α-terpinene in sage essential oil (0.2 to 0.6%).

Packing also affects product quality. An examination of films used for packaging of dried sage tissues (Paillard, 1994b) has shown that increasing thickness reduces film permeability to volatile substances thus maintaining the quality of the plant material for longer. For a given thickness, polypropylene is less permeable than polyethylene. Other characteristics of the films, such as resistance to mechanical strength, thermosealing properties, uv- and light-penetration may also be important.

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8. GENETIC IMPROVEMENT OF CULTIVATED SPECIES OF THE GENUS *SALVIA*

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INTRODUCTION

While the utilisation of species belonging to the genus *Salvia* has a great tradition, their large scale cultivation only started in the 20th century. This can be well demon strated by the story of *Salvia sclarea*. Although the species can be found widely distributed throughout the Southern regions of the former Soviet Union, it was not until 1922 that it was introduced as a crop for oil production (Zinchenko, 1960). It was reported, that for the next four years, the hectareage and oil production grew to 9500 hectares and 24 metric tonnes. The first attempts at large scale production of the above mentioned species in the USA can be dated to the mid-1940s and by the mid-1990s North Carolina had become the leading producer of clary sage oil in the United States (Lawrence, 1994). Similarly in Europe, the large scale cultivation of S. *sclarea* only started in the second half of the 20th century, (France, Hungary, Bulgaria etc.). For instance the although plant was introduced into Bulgaria in 1940 from France (Zheljazkov *et al.*, 1996), its large scale cultivation was initiated 14 years later near Sofia and around Plovdiv.

The other important cultivated species of the genus, *Salvia officinalis* has a very similar history, although grown in past centuries in small gardens in Europe its large scale cultivation started only recently.

The cultivation of the species requires an adequate cultivation technology includ ing appropriate plant material (Bernáth, 1996). At the beginning of *Salvia* species cultivation indigenous local, or introduced populations were used, with moderate efficacy. To create a base for economical production, the breeding work especially on *S. sclarea* and *S. officinalis* became a more intensive one. Completing the data of Franz (1996) the selection work oriented to the members of the genus are presented in Table 1. Based on the collected information the main selection goals in the genus are as follows:

a) getting cultivars of high productivity (for both dry matter and secondary compounds),
b) making cultivars with appropriate compositional character

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Table 1 Recent efforts on genetic improvement of the species of genus *Salvia,* by countries.

Species	*Country*	*Goal of genetic improvement*	*References*
S. officinalis	Austria	cold resistance, decrease of – thujone content	Franz 1990, Franz 1996,
	France	2–3% oil, 40% thujone	Franz 1996,
	Hungary	high essential oil , low α-, and β-thujone	Franz 1996,
	Israel	high yield and essential oil, max. 50% thujone and 10% camphor	Franz 1996, Putievsky *et al.* 1990,
	Italy	high essential oil, synthetic variety	Bezzi 1996, Landi and Bertone 1996,
	Spain	changes of oil composition by hybridization	Sanchez *et al.* 1995
	Switzerland	cold resistance	Franz, 1996,
	Bulgaria	mutant lines with 0.43% essential oil, male sterile lines	Dascalova 1986, Mekhraz *et al.* 1988,
S. sclarea	Hungary	selection of annual form, high essential oil and sclareol content, cold resistance	Franz 1996, Zámbori-Németh and Tétényi 1990,
	Israel	high yield and essential oil, producing hybrid vigor	Elnir *et al.* 1991a, Elnir *et al.* 1991b
	Russia	producing inbred lines of high essential oil	Goncharyuk *et al.* 1988,
	Ukraine	making hybrids and annual forms of high productivity, producing male-sterile, haploid and inbred lines, essential oil up to 2%, disease resistance	Arinshtein *et al.* 1985, Bugara and Rusina, 1989, Bugayenko *et al.* 1995, Kovalev 1989, Pogorelskaya and Reznikova 1986, Rusina *et al.* 1997, Zobenko and Arinshtein 1989, Zobenko *et al.* 1989, Vlasova 1986,
S. aethiopis	Ukraine	making hybrids with *S. sclarea*	Rusina *et al.* 1997,
S. fruticosa	Greece	high and homogenous essential oil, min. 40% cineol, low α-, and β-thujone content,	Franz 1996, Skoula *et al.* 1994
	Israel	high yield and essential oil, making hybrid with *S. officinalis*	Franz 1996, Putievsky *et al.* 1990,
S. grandiflora	Ukraine	making hybrids with *S. sclarea*	Rusina *et al.* 1997,
S. lavandulifolia	Spain	making hybrids with *S. officinalis*	Sanchez *et al.* 1995
S. scabiosifolia	Ukraine	making hybrids with *S. sclarea*	Rusina *et al.* 1997,
S. tomentosa	Israel	making hybrid with *S. officinalis*	Putievsky *et al.* 1990,

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c) changing or stabilising the life form of the species,
e) improving cold resistance,
f) getting cultivars with disease resistance

These selection goals are approached by different strategies and methods from country to country, depending on the local tradition and experiences. However, it is a common phenomenon that the high morphological and chemical diversity of the species are utilised, even if the plant is growing wild locally, or that the indigenous populations have been introduced from exterior habitats.

MORPHOLOGICAL AND CHEMICAL DIVERSITY OF INDIGENOUS POPULATIONS AS A BACKGROUND FOR GENETIC IMPROVEMENT

It is obvious, that the accumulation level and composition of essential oils, even in the members of the Lamiaceae family is dependent on both the developmental stage of the plants and the environmental conditions of growing. This phenomenon can be observed in the case of *Salvia* species, too. Referring to the literature data the essential oil content of *Salvia sclarea* (summarised by Lawrence, 1994) and *S. officinalis,* like most members of the oil-reach group of the genus, reaches its maximum values at full flowering, because the calyces contain by far the most essential oil glands per unit area. However, in the case of *S. sclarea* only slight changes were found by Bankovic *et al.* (1993) between flowering and seed setting phases in the quantitative and qualitative composition of the oil. There are many examples of the effect of external factors, too. By the results of Ilieva and Bhattacharyya (1990) the altitude of the growing may effect both the accumulation level and composition of oil in *S. sclarea.* The essential oil content was higher in plants growing at lower altitudes (at 380 m at Kazanlik), where the linalyl acetate content became moderate (53.8–68.5%). Growing three identical *S. officinalis* clones in different ecological circumstances of Italy, it was proven by Bezzi (1996), that both the accumulation level and the composition of the essential oil had changed. By analysing four species of the genus *(S. officinalis, S. sclarea, S. austriaca, S aethiopis),* the ecological flexi bility of essential oil accumulation was determined by us (Bernáth *et al.,* 1991). Growing plants under natural Hungarian conditions and phytotron chambers, it was stated that under field conditions and in a warm program with high illumuina tion the accumulation of essential oil was promoted, universally. On the other side, the cold temperature and poor light resulted in a remarkable reduction. Universal trends were observed in the changes of the oil composition. By the increase of light intensity the accumulation of borneol and camphor was promoted in both *S. officinalis* and *S. austriaca.* On the contrary, the lack of light resulted in lower accumulation levels of cubebene and cis-cariophyllene in *S. sclarea* and *S. aethiopis.* In spite of these changes, the magnitude and spectra of accumulation proved to be a characteristic mark of the species, and populations. However, it seems to be obvious, that much more attention, and more detailed investigation is required if we want to make a distinction either on intraspecific or population level.

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There are numerous publications which prove the existence of the high morphological and chemical diversity of the species of *Salvia* genus.

The high compositional diversity of essential oil samples produced from *S. sclarea* of different origin can be explained by its wide geographical distribution (Lawrence, 1994, Boelens, 1997). The chemical diversity of the species is well demonstrated by the data presented in Table 2. However, new chemotypes are found day by day analysing the indigenous local populations (Souleles and Argyriadou, 1997). The large compositional differences are obvious, in spite of the fact, that the sampling of the plants, extraction and analysis of the oil have been done differently, from country to country.

The chemical diversity of S. *officinalis* has been proven by many authors, too. Some examples are given in Table 3. The chemical diversity of the wild or/and reserved populations are very high and it provides a promising background for starting selection.

From the selection point of view the statement of Putievsky *et al.* (1992) has to be considered. Seeds of wild growing *Salvia officinalis* populations were gathered along the Adriatic coast, and cultivated afterwards in Israel. The morphological features, essential oil content and composition of these plants were found to be similar to plants growing in the wild. It was shown in both natural and artificial conditions, that in conjunction with earlier investigations (Vokou *et al.,* 1977, Ivanic *et al.,* 1978, Gruznov *et al.,* 1981) plants from north and south Dalmatia have a similar compositional character (27% thujone, 30–33% camphor), while plant populations from central Dalmatia differ much (47% thujone, and less than 14% camphor). It was stated, based on the results, that many of the morphological characteristics, and especially the essential oil composition are under genetic control. In addition, a large intra-population variability (as much as 45%) was found in some of the characters, which provide appropriate conditions for further selection of individuals.

The existence of the high chemical diversity was proven in the case of *Salvia fruticosa,* too (Putievsky *et al.,* 1986b; Skoula *et al.,* 1994). The plant which is endemic in Eastern Mediterranean region is very rich in essential oil, ranging from 2.5% to 6.0%. Each of the populations can be characterised by intensive accumulation of 1, 8-cineol (30% to 50%), but the other essential oil constituents vary either on inter-, or intra-population level. Among populations, the presence of *ß*-pinene, l-phellandrene, *a*-terpineol and terpinyl acetate may vary, while at intra-population level the presence of camphene, sabinene, *α*-, and *ß*-thujone, camphor and borneol considered to be specific.

For effective breeding, knowledge on the inheritance patterns for at least the most important plant characteristics would be necessary. However, in case of the *Salvia* species, the information on genetical regulation is rather poor or at least very rarely published.

IMPROVEMENT OF POPULATIONS BY SELECTION

The cultivation of the species belonging to the genus *Salvia* was started by selection and introduction of wild growing populations. Thus selection has remained one of the most

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Table 2 Compositional differences of clary sage oil produced in different regions of its cultivation area.

Main components (%)	*Uzbekistan (1)*	*France (2)*	*U.S.A (2)*	*Israel (3)*	*Russia (3)*	*Bulgaria (4)*	*Spain (5)*
myrcene	0.2–0.8	–	–	–	–	0.9–1.6	1.6
p-cymene	0.0–0.1	–	–	–	–	–	–
limonene	0.0–0.1	–	–	–	–	0.9—-1.8	–
linalool	**22.0–36.0**	10.0–20.0	25.0	1.7	**31.0**	9.4–10.9	**32.9**
α-terpineol	6.4–12.0	0.5–2.5	4.5	0.3	10.3	–	5.6
neral	0.0–4.5	–	–	5.9	0.1	–	–
linalyl acetate	25.0–51.0	**60.0–70.0**	**55.0**	0.3	35.0	**76.8–84.8**	16.9
nerol-geraniol	0.0–12.0	t – 1.5	2.5	–	–	–	1.8
neryl acetate	1.5–3.2	0.3–1.0	2.8	3.0	2.6	0.2–0.3	1.2
geranyl acetate	2.9–5.4	0.5–1.8	2.8	**38.3**	5.4	0.1–0.5	1.6
β-caryophyllene	0.3–1.8	1.5–2.5	0.7	–	–	–	4.8
germacrene D	–	3.0–5.0	1.8	0.8	2.7	–	**7.6**

(1)Dzumayev *et al.* 1995, (2) Lalande 1984, (3) Elnir *et al.* 1991a, (4) Mehraz *et al.* 1988, (S) Torres *et al.* 1997.

Table 3 Compositional differences of essential oil produced from *Salvia officinalis* of different origin.

Main components (%)	*Slovenia (1 a)*	*Slovenia (1b)*	*Italy (2)*	*Israel (3)*	*Spain (4)*	*Hungary (5)*	*Slovakia (6)*
α-thujone	10.7	9.9	**39.3**	**55.0**	**22.8**	**23.9**	
β-thujone	2.6	3.2	3.0	10.0	4.3		**35.0**
limonene	2.7	2.7	0.7	–	0.8	–	1.1
1,8-cineole	3.5	4.0	7.7	**13.0**	**15.7**	6.2	7.9
camphor	12.8	**14.3**	2.1	2.0	5.0	6.9	**14.2**
β-pinene	–	–	7.2	1.0.	8.9	3.3	2.1
α-humulene	–	–	12.4	–	0.3	–	–

(1) Baricevic *et al.* 1996, a-autochtonous population, b-prealpine region, (2) Pace and Piccaglia 1995, (3) Putievsky *et al.* 1990, (4) Sanchez-Gomez *et al.* 1995, (5) Bernáth *et al.* 1991, (6) Hollá and Vaverková 1993.

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effective tools up to present day, even it is used exclusively (Bezzi, 1996; Bugayenko *et al.,* 1995, Franz, 1990, Skoula *et al.,* 1994), or combined with other breeding methods (Putievsky *et al.,* 1990, Mekhraz *et al.,* 1988, Landi and Bertone, 1996).

The use of individual selection from wild-growing *S. sclarea* populations resulted in valuable initial material for many lines of genetic improvement (Bugayenko *et al.,* 1995). The first cultivars in the former USSR were developed by Maychenko (1961) using continuous individual selection method. The first Bulgarian cultivars "Iskra" and "Bisser" were developed also by the same method (Ilieva, 1980). Based on the activity of the Ukrainian scientific group, biotypes with different life forms (annual, biennial) were developed afterwards (Zobenko, 1968, 1972), including early ripening, mid-ripening and late-ripening, winter-hardy, high yielding forms with high oil content. Thus as a result of systematic individual and negative selection for early ripeness, the sage variety "Crimsky ranniy" was bred. The selection was effective in developing materials of disease resistance, too. It was reported by Savchenko and Plis (1990) that the population developed by selection method proved to have high resistance against *Thielaviopsis basicola,* and also a high oil content and good quality essential oil.

Selection is commonly applied to *Salvia officinalis* for its genetic improvement. It means also an advantage, that this species can be very easily propagated both vegatatively, by cuttings and generatively by seed sowing. Methods were developed for making single plant selection by sowing seeds (Putievsky *et al.,* 1986a), or using stem cuttings as well (Raviv *et al.,* 1984). An effective long term selection work was carried out in co-operation by Austrian and Italian scientists (Franz, 1990; Bezzi, 1996). The compression test of 12 clones over three years has shown large

Table 4 Production and chemical characteristics of selected *Salvia officinalis* clones in the mean of three years of investigations, 1989–1991 (Bezzi 1996).

Clones	*Biomass (t/ha)*	*Essential oil (ml/100 g)*	*Composition of essential oil (%)*			
			α-thujone	*β-thujone*	*euchalyptol*	*camphor*
Fl 1	4.2	0.29	31.66	4.50	13.11	10.05
Fl 4	3.6	0.32	31.21	2.80	15.98	7.80
Fl 7	5.4	0.29	21.82	**1.59**	3.55	18.24
Fl 10	3.3	0.32	25.49	3.17	5.82	28.48
FL12	5.4	0.26	23.05	**1.66**	7.23	13.96
Fl20	2.9	0.31	**3.51**	9.12	2.84	16.75
TN 58	5.4	0.27	28.16	21.53	9.50	10.22
TN 75	5.6	0.36	46.12	6.69	2.01	17.15
TN 132	6.0	0.23	24.82	4.84	18.73	10.10
TN 191	6.4	0.31	37.64	3.33	13.54	6.64
TN 600	5.8	0.27	45.10	3.61	10.71	10.28
TN B	3.6	0.31	**2.58**	**1.40**	12.69	26.32

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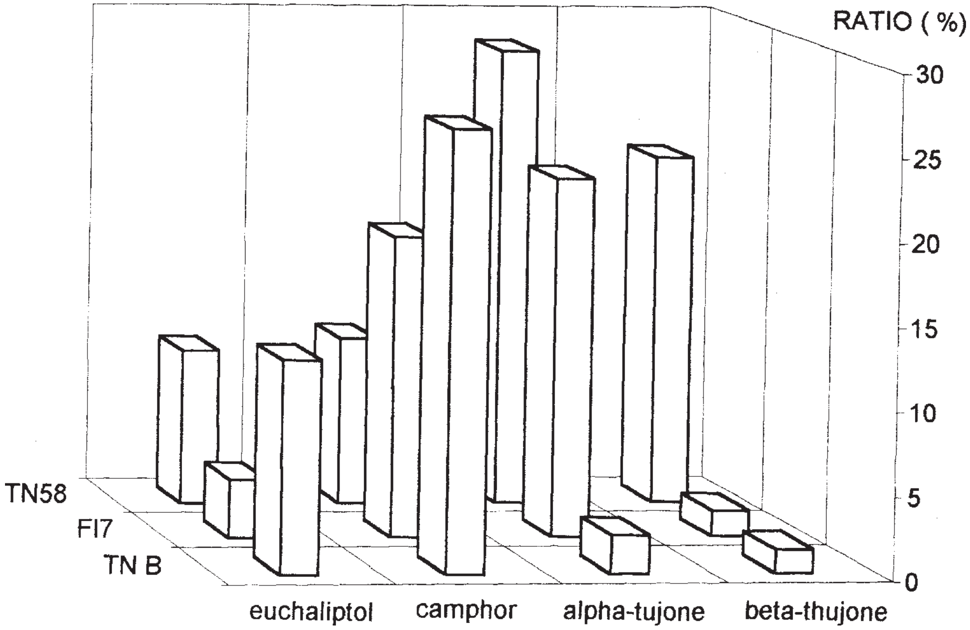

Figure 1 Chemical diversity of *Salvia officinalis* clones achieved by continuous selection (Bezzi 1996).

differences among their biomass and essential oil production (Table 4). There were clones TN 191, TN 132 and TN 600 characterised by high biomass production, while others (TN 75, Fl 10, and Fl4) were outstanding in essential oil accumulation, showing 0.36%, 0.33% and 0.32%, accumulation level, respectively. The majority of the clones showed high—thujone content (21.82–46.12%) with the exception of clones Fl 20 and TNB in which the accumulation level is below 4%. Much more attention has to be paid to the *ß*-thujone level, a compound which has been stated as being hazardous to health. In this respect the advantages of the clones Fl7, Fl12, and TNB has to be emphasised, as they accumulate less than 2% of *ß*-thujone. The chemical diversity could be increased and fixed by continuous selection and special strains were developed (Fig. 1).

The possibility of the application of biotechnological methods in the multiplication and stabilisation of selected *Salvia officinalis* clones was described by Azzilotti in 1987. A mother plant was chosen by its superior perfume and mor phological properties and multiplied using micropropagation. A percentage of 91% of rooting was obtained and the morphological and chemical properties of the clones remained unchanged after four generations. As *S. officinalis* is a perennial species, this method may contribute to the quick propagation and acceleration of the cultivars distribution. However, this practice is not commonly used yet.

The advantages of the selection were justified in the case of the other species of the genus, too. It was stated by Skoula *et al.* (1994) that the investigation of the natural diversity of *Salvia fruticosa*—oyer its theoretical value—contributed to the

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improvement of propagation material. It was emphasised by the authors, that selected clones of *S. fruticosa* should be cultivated in the Mediterranean regions in order to obtain crops with homogeneous essential oil and stable biomass production.

CREATION OF NEW CULTIVARS BY HYBRIDISATION

Interspecific Hybrids

Although there is a few data on the interspecific hybridisation of *Salvia* species, the application of interspecific crossing might have much more importance in the future based on the outstanding results of the first attempts. Until now, mainly wild growing species have been crossed with *S. officinalis* and *S. sclarea* in order to bring useful characteristics into the cultivated species.

It was reported by the Ukrainian research group (Bugayenko *et al.*, 1995) *that S. grandiflora* Etling. and *S. aethiopis* L. can be applied as donors for clary sage building up resistance against environmental stress and diseases. Thus, the method of embryo culture was developed to transfer the above mentioned characteristics into clary sage and thereby extend genetic variability by somatic hybridisation. The interspecific hybrids created by manipulation of embryo cultures were included in the selection process and unique genotypes were obtained exceeding the varieties available in Ukraine in both yield and oil content.

The existence of interspecific hybridisation was reported in the case of other *Salvia* species. Spontaneous hybridisation of *S. officinalis* and *S. fruticosa* was described by Putievsky and Ravid (1984) and by Putievsky *et al.* (1987). By growing the two species nearby in the same research garden spontaneous crossings were observed. In respect of both yield and essential oil production intermediate charac ters were shown by the spontaneous hybrid plants. Some years later interspecific crosses were made between the above mentioned species experimentally (Putievsky *et al.,* 1990), involving another Mediterranean species, *Salvia tomentosa* Mill. The clones used for crossing were obtained after several years of single plant selection. As a result of crossing of *S. officinalis* and *S. fruticosa* a good cross-compatibility was found, but in combinations with *S. tomentosa* the crossability was low: zero to 2% in the cross with *S. officinalis* and zero in the cross with *S. fruticosa*. Moreover, a large number of these seeds did not germinate, some plants died at the seeding stage, while others did not reach flowering. Pollen fertility and seed set studies revealed that there are reproductive barriers among these species. However, as some of the hybrids were at least partly fertile, it seems to be possible to use wild species the gene pool in the genetic improvement of *S. officinalis*. No further information is known about the success of this work. However, in the case of *S. officinalis,* breed ing is long term work due to its perennial life form, and even the discussed method of crossing with several back-crosses needs considerable time before practical results are obtained.

The comparison of essential oil contents of the parents and hybrids in the above experiment shows a special picture, which is demonstrated in Table 5. In the essential oil of *S. fruticosa* and *S. officinalis* hybrids there is a medium level of 1, 8-cineole,

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Table 5 Crossability and oil composition of three *Salvia* species and their hybrids (Putievsky *et al.* 1990).

Parents	*Crossability (%)*	*Seed set (%)*	*Main compounds of essential oil (%)*						
			α-pinene	*β-pinene*	*1,8-cineole*	*α-thujone*	*β-thujone*	*camphor*	*β-caryophyllene*
S. officinalis	85	65	1	1	13	55	10	2	0
S. fruticosa	92	37	6	11	48	0	0	8	8
S. tomentosa	96	40	2	2	19	0	59	1	0
S.officinalis x S. fruticosa	36	9	3	7	30	27	7	4	4
S. fruticosa x S.officinalis	34	9	3	7	24	29	7	4	2
S. tomentosa x S. officinalis	2	4	2	3	12	49	6	4	2

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α- and *ß*-thujone, camphor, *ß*-caryophyllene, *α*- and *ß*-pinene. However, the oil of the hybrid between *S. tomentosa* and *S. officinalis* is very similar to that of *S. officinalis* and there is no increase of *ß*-thujone because of the expected influence of *S. tomentosa*.

Mainly the intermediate heritability character of essential oil composition was proved also by Spanish experts (Sanchez-Gomez *et al.,* 1995) making hybrids between *S. officinalis* L. and *S. lavandulifolia* Vahl. ssp. *vellera* (Cuatr.). While the major components of *S. officinalis* were a-thujone (22.82%), 1, 8-cineole (17.71%), viridiflorol (10.92%) and camphor (14.63%), the oil of *S. lavandulifolia* contained 1, 8-cineole (43.73) and camphor (14.63%). In addition to possessing intermediate amounts of most components of both oils, the hybrid accumulated a-thujone and *ß*-thujone (3.04%, 0.56%) and manool (1.94%) which were the characteristic compounds the *S. officinalis* and not found in *S. lavandulifolia*.

Intraspecific Hybrids and Heterosis Breeding

Intraspecific hybridisation is widely used in the genetic improvement of *Salvia* species. The most important achievements in the case of clary sage are reported here. The hybridisation work of Elnir *et al.* (1991b) is of great importance first of all from a theoretical point of view. Wild cultivated chemotypes of *S. sclarea* originally from Israel and Russia which differed in oil composition were crossed to study the inheri tance pattern and to gain new oil composition. The essential oil distilled from the Israeli type had a citral-like odour, mentioned as a citral chemotype while the plant material of Russian origin produced the commercial type of oil. Reciprocal hybridis ation of the chemotypes resulted in two different types of hybrids, which shows the role of maternal effects. One of the hybrid groups accumulated linalool, geranyl acetate and geraniol in a relatively high amount, 22.0%, 22.5% and 22% respectively, while in the other one the presence of linalyl acetate and geranial was relatively high (12.6% and 7.2%). Besides, most of the components of the essential oil of the hybrids were intermediate between those of the parents. However, it seems, that in this case the existing intraspecific chemical variability can not be used effectively for the establishment of special cultivars, because the hybrids proved to be completely sterile, despite the observed normal meiosis (Elnir *et al.,* 1991a).

Considerable practical results were achieved however by hybridisation of clary sage lines in Ukraine and Bulgaria. To get cultivars of high productivity, hybridisation was started in the early sixties simultaneously in both countries. In the Bulgarian breeding program the local cultivars were hybridised with the best Ukrainian types to get progenies of high productivity and cold resistance (Ilieva, 1980). As a result of the long process of selection four cultivars were developed: "Lazour", "Slunchev luch", "Roza" and "Zarya". All these cultivars surpassed the standard populations in essential oil content (which ranged between 0.20–0.24%) and in oil yield. The new cultivars were distinguishable by morphological characteristics and the time and duration of anthesis.

In the Ukraine (Zobenko *et al.,* 1989, Bugayenko *et al.,* 1995) the main aim of breeding *S. sclarea* was to get varieties of high productivity, populations owing

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different developmental cycles (annual and biennial) and plant material with resistance again root rot (*Thielaviopsis basicola* (Bek. et Br. Ferraris). By intraspecific hybridisation (between cultivars) using an effective castration method new clary sage cultivars, the annual "Crimsky odnoletniy" and "Crimsky ultraskorospely" charac terised by a very short vegetation period were bred.

In the above mentioned two countries, the effectiveness of breeding could be increased even by application of heterosis breeding. It was proven, that by means of inbreeding, uniform and highly productive lines could be developed (Goncharyuk *et al.,* 1988). Inbreeding depression is affected by the genotype of the source variety and is, in several cases, not considerable. By continuous inbreeding Vlasova (1986) produced lines differing in both morphological and production-biological characters from the original cultivar. Even the accumulation level of essential oil increased up to 2%. Beside F1 hybrid production, these lines proved to be useful for other breeding methods, such as simple selection or synthetic varieties. Inbreeding combined with multiple selection recently led to practical results. A new late ripeness cultivar of clary sage under the name "Crimsky pozdniy" was created and introduced into large scale cultivation (Zobenko, 1995).

In *Salvia* species, castration is not an economically acceptable method for the effective heterosis breeding. In large scale seed production there is a need for male sterile counterparts. It seems, that in the case of *S. sclarea* this cannot be a problem. The occurrence of male sterility had been found—in accordance with *S. nemorosa* and *S. officinalis* (Mohan and Kans, 1990), which may contribute to the effectiveness of the hybridisation. Male sterile plants were found in Bulgaria, too (Dascalova, 1996), in a collection of freely pollinated lines and among individuals of the "Boyana" cultivar. By studying the biology of male sterility it has been established that male sterility increases the quantity of seed production compared with the seed set ratio of the fertile plants by an average 20%, due to sterilisation processes in the different stages of megagametogenesis and embryogenesis. It may even increase the value of using male-sterile plants in the heterosis breeding.

In Ukraine, cytoplasmically male sterile analogues of the breeding lines have been developed and are used for seed production (Arinstejn *et al.,* 1985; Kovalev *et al.,* 1988; Kovalev, 1989). It was established, that the ratio of the pollen donor parent should be at least 17–25% compared to the male-sterile mother plants. However, both in the production of hybrid seeds and in maintenance of the lines, the pollina tion will be complete only in the presence of bee families.

There has been much less experience in the intraspecific hybridization of the other *Salvia* species when compared to *S. sclarea,* as discussed above. However, the results achieved by the co-operation of Italian-Austrian groups on *S. officinalis* are worth a mention (Franz, 1994; Bezzi, 1996; Landi and Bertone, 1996).

In order to achieve successful breeding and cultivar development and by realising the lack of knowledge in this context, studies on the inheritance of the most impor tant plant traits by crossings, were carried out (Franz, 1994). It is obvious from the data presented in Table 6. that crossing of cineol type (P132) and a-thujone type (P191) plants resulted in both α-thujone and ß-thujone type individuals, as well. Also it is a very interesting phenomenon, that while both parents contain relatively large

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Table 6 Compositional segregation of hybrids made by crossing of different *S. officinalis* chemotypes (Franz 1994).

Genotype	*Main constituents of the essential oil (%)*					
	Cineol	*α-thujone*	*β-thujone*	*camphor*	*α-pinene*	*camphen*
P132	25.89	29.66	8.09	13.08	4.62	2.52
P191	17.16	34.66	6.87	13.88	8.57	4.72
132 × 191.14	**33.06**	0.20	**20.77**	10.26	**21.34**	3.92
132 × 191.13	**32.19**	0.50	**24.68**	15.66	**9.12**	4.64
132 × 191.19	23..29	22.94	6.44	14.37	13.85	6.85
132 × 191.15	22.06	27.76	6.10	11.31	13.98	3.83
132 × 191.15	18.49	25.82	5.49	18.70	12.24	5.72

amounts of a-thujone (29.66–34.66%), its amount decreased to below 1% in one group of hybrids. The same unexpected trend of changes can be observed in the case of the majority of the other compounds. Based on the results of the above experiment, it was admitted by the author, that additive or epistatic gene effects should be present in the regulation of formation of essential oil compounds.

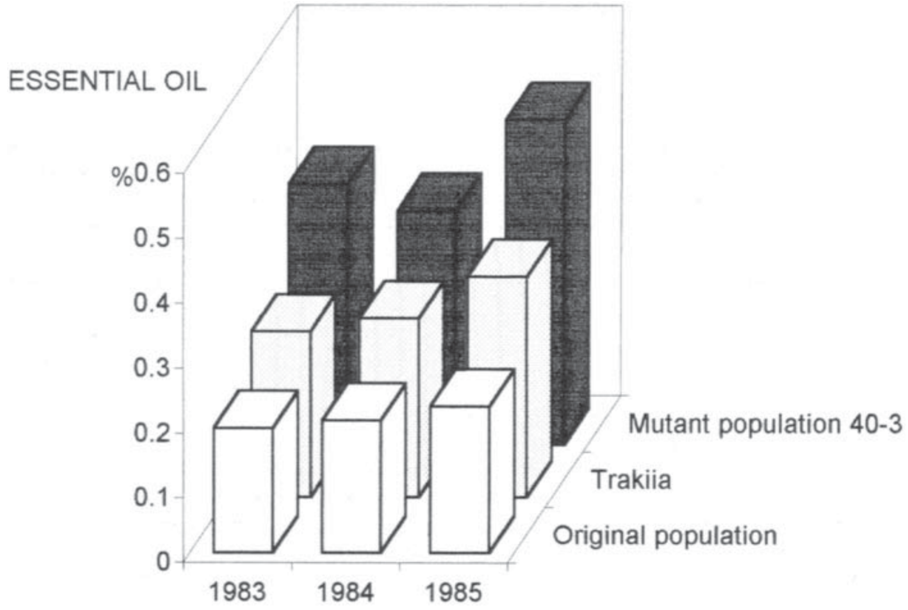

Figure 2 Accumulation level of essential oil in the original population of Sa*lvia sclarea a*nd cv. “Trakiia” as well as in the mutant line number 40–3, in a three year experiment (Mehraz *et al.* 1988).

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In the case of this species, instead of heterosis breeding, establishment of synthetic varieties seemed to be more advantageous, due to the biological background and high costs of Fl hybrid production. In practice, the polycross method can be easily applied, considering the good vegetative propagation possibilities *of S. officinalis*. For cultivar development, testing the combining ability in diallele trials, selection of lines of proper homogenity, large leaf area, considerable essential accumulation level and high productivity was carried out. The results of this work and the establishment of a synthetic variety in polycross blocks from these clones were reported by Bezzi (1996) as well as by Landi and Bertone (1996).

POLYPLOID FORMS AND MUTATION BREEDING

It is only in the last ten years, that it has been proven that the induction of mutant and polyploid forms may contribute to the improvement of the genetic background of *S. sclarea*. The construction of these mutant lines being carried out by colchicine treatment.

Based on the results of Savchenko (1990), the induced polyploids can be well characterised and selected from the basic population according to an index. This index is calculated by taking into account the leaf shape, its margin, colour, surface and the number of germination pores in the pollen grains just before flowering time.

The mutation breeding produced even practical results. Mekhraz *et al.* (1988) constructed a new cultivar producing mutants by a 24 h treatment with colchicine. The growth, development and essential oil content of mutant lines were studied and compared with both the standard cv. “Trakiia” and the initial population. It was found that the new lines had faster growth and blooming rates. Moreover, some of the lines (especially line number 40–3) produced much higher total fresh mass and essential oil yield. The accumulation level of essential oils in this special line mentioned under the name “Boyana” proved to be superior to the standards in three year experiments (Fig. 2). The essential oil composition of the selected cultivar is characterised mainly by the presence of linalyl acetate (76.1–84.83%), linalool (9.36–10.88%) and limonene (0.87–1.80%).

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9. THE ECOPHYSIOLOGY OF *SALVIA:* DISORDERS AND ADAPTATION

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INTRODUCTION

Since only a few sage species are being intensely cultivated, there is a rather scarce, scientifically documented information on the interaction of *Salvia* plants with different environmental components, in particular stress factors potentially related to physiological disorders. In the present chapter several aspects of the ecophysiological adaptation of various *Salvia* species are reviewed, such as drought, heavy metal and herbicide tolerance and the physiology of seed germination. Furthermore, a descrip tion is given on the available data on the growth competition between plants of the same species as well as among different species.

DROUGHT TOLERANCE

Some species of coastal sage and chaparral shrubs of California are extremely tolerant of tissue dehydration, surviving water potentials as low as -9MPa during dry summer months. Such low water potentials (high tensions on xylem water) are known to cause severe embolism formation in the xylem vessels of woody plants, blocking water transport and potentially causing shoot dieback. Thus drought-hardy species of coastal sage and chaparral are either extremely resistant to water stress-inducted embolism or they become severely embolized during summer drought. An estimation of susceptibility to water stress-inducted embolism indicated that 50% loss in hydraulic conductivity would occur at -4.5 MPa for *Salvia mellifera*. Irrigation of *S. mellifera* for one summer reduced loss in conductivity from 78 to 38% and increased leaf areas 10-fold, indicating that xylem embolism and leaf drop were drought inducted. Results show that xylem tissues of *S. mellifera* are more sensitive

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to water stress and tissue dehydration than those of co-occurring *Ceanothus megacarpus* (chaparral). The observed ability of *S. mellifera* to inhabit drier sites than *C. megacarpus* may result from drought deciduousness in summer and high growth rates in spring that facilitate the rapid construction of new xylem and leaf tissues. It may be that facultative drought deciduousness in coastal sage is tightly coupled to drought-inducted embolism of xylem tissues (Kolb and Davis 1994).

In another report on *Salvia mellifera* G., Hargrave *et al.* (1994) came with interesting conclusions while investigating the relationship between conduit (vessel and tracheid) diameter and water-stress-inducted air embolism using a double staining technique, between irrigated control plants at water potential of -1.3 MPa and water stressed plants at about -8 Mpa. More specifically, water stress was inducted either by natural drought conditions or by laboratory drying of shoots from previously irrigated shrubs. Diameters of non-embolized and embolized conduits were then measured microscopically in transverse stem sections. In irrigated controls there was little embolism and mean diameters were not significantly different for embolized vs. non-embolized conduits. For both artificially dehydrated and naturally droughted plants there was a 91% drop in kh due to embolism and the mean diameter of embolized conduits was about 30 um vs. 21 um for non-embolized conduits. With increasing conduit diameter there was an increasing probability of embolism. Wider conduits may have larger pores in their pit membranes, thus increasing their vulnerability to water-stress-inducted embolism. Alternatively, wider conduits may merely have more pits, thus increasing their statistical chances of having particularly large pore in an air-exposed pit membrane. Narrow vessels and tracheids provide an interwoven auxiliary transport system that appears to be of importance to transport when many of the wider, more efficient conduits become embolized.

Researchers working with *Salvia reflexa* Hornem. (Weerakoon and Lovett 1986) grown in pots of soil and subjected to drought treatments in glasshouse conditions, mention that quite short durations of water stress significantly decreased leaf area, top and root dry weight. The plants rapidly adjusted the water loss below potential evaporation rates. They survived and recovered from periods of drought for up to 30 days. However, dehydrated plants did not attain the growth rates of undroughted plants, even after short drought periods. Referring to the same species, Weerakoon and Lovett (1986) suggest that the rate of germination and total germination were decreased as the osmotic potential of germination medium increased from –0.4 to -1.4 MPa.

HEAVY METAL TOLERANCE

Zheljazkov and Zheljazkova (1996) investigated the simultaneous effect of soil heavy metals on the essential oil content and productivity of *Salvia sclarea* L. as well as the heavy metal accumulation in plant parts (roots, stems, inflorescence). As reported, the simultaneous contamination of soil (taken at a distance of 0.5 km from the source of pollution) with excessive amounts of Cd and Zn decreased the yield of fresh inflorescences by 14–19% and the yield of oil by 12–18%. There was no

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contamination of the oil and no visible toxicity injuries. Also, a cultivar response to Cd, Cu and Mn was determined. In most cases, Cd concentration in the plant parts was in the order: leaves > roots > inflorescence > stems and the concentration of Mn, Cu and Zn in the order: leaves >roots > inflorescence > stems, while the concentration order of Fe was leaves=roots > inforescence=stems. In a similar study, Zheljaskov and Nielsen (1996) came to the conclusion that clary sage can be grown on sites of severe air and soil heavy metal pollution as a substitute for some other edible crops.

Chromium toxicity in *Salvia sclarea* L. has also been investigated (Corradi *et al.* 1993), in respect of its effects on seed germination and seedling development. Seeds and seedlings of the plant were treated with different concentrations of hexavalent chromium ($K_2Cr_2O_7$). *In vitro* seed germination was not affected, but when the emergent radicle came into contact with the Cr solution, its growth was inhibited although early shoots and cotyledons developed normally. If the seedlings were transferred to Eppendorf vials so that the root were completely immersed in the Cr solution, not only root elongation, but also shoot and cotyledon development were inhibited. After 48 hours, cotyledons appeared chlorotic and chlorophyll and carotenoid contents were reduced, but chloroplast ultra-structure was normal. The only ultrastructural alteration was a partial detachment of the plasma membrane from the cell wall.

PHYSIOLOGY OF SEED GERMINATION

Effects of Light and Temperature

A great deal of work has been done in the field of optimizing the conditions for maximum sage seed germination. Temperature and light are the main factors investigated. Most reports concern both factors.

A work aiming at determining the temperature minima, maxima and optima for seed germination of the species *Salvia officinalis* and *S. sclarea* taking into consideration the role of light in the germination process (Oberczian and Bernath 1988), showed that the optimum constant temperature for *S. officinalis* germination was 25 °C, while among the varying temperatures the best program was 30/20 °C day/night. At low temperatures (e.g. at 5 and 10 °C) germination was retarded, slow and unambiguously inhibited. The 35 and 40 °C values above the optimum caused heat-inducted damage, thus 40 °C could be considered as the upper temperature limit. The germination of *S. officinalis* was markedly influenced by the light : below 20 °C it was inhibited., above 20 °C the process was stimulated. The temperature extrema of the germination of *S. sclarea* were identical with those of *S. officinalis*. Its optimum temperature point was lower, having a value between 15 and 20 °C. The germination-stimulating effect of day/night varying temperatures was not observed. The role of light in the case of *S. sclarea* was not remarkable. Some retardation was observed but only expressed in terms of the germination percentage.

Another report concerning temperature and light interactions on the germination of seeds of *Salvia hispanica* L., (Labouriau and Agudo 1987b), gave the following results: 1) The synchronization of germination responds to temperature, but not to

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light, 2) Temperature-light interactions are very different in connection with germinability and with germination rates, 3) These seeds are physiologically heterogeneous, containing positively photoblastic subpopulations at 15 °C and negatively photoblastic subpopulations at 35 °C; between 20 and 31 °C germination is light indifferent, 4) Red light flashes and far-red light flashes produce opposite effects on germinability, both at 15 and 35 °C, 5) The photoplastic germination at 15 °C is light triggered and not light driven, 6) Effect on a single light flash on the germinability at 15 °C could be reverted, at least in part, by a subsequent flash of far red, but no such effect was observed on the germination rate.

At this point, we must mention a previous work of Labouriau and Agudo (1987a) concerning the germination of seeds (*S. hispanica* L.) produced at Altos de Pipe, near Caracas (Venezuela), at 1500 m elevation, from an original batch of Mexican origin, under standardized light conditions (a flash of white incandescent+fluorescent light every 8 hours, involving only the temperature factor). The experiments were done with one-year-old seeds, which showed a viability from 67.1 to 72.8%. With larger storage periods viability dropped significantly. The isothermal incuba tion of fine samples of 200 seeds at each of 33 different temperatures was performed. Observations were made every 8 hours, using the germination criterion of the radicle emergence. In detail, the extreme germination temperature limits were 3.3 °C +/-0.4 °C and 39.8 °C +/-0.4 °C. From 3.3 to 15.0 °C and from 25.7 to 39.8 °C the germination process was limited by the germination capacity; from 25.0 to 15.7 °C, by the germination rate and in this last range there was no dormancy. The Arrhenius graph of the rate was curvilinear, with its minimum at the optimal range of the rate. Below 11.3 °C and above 35.2 °C germination may be limited by partial processing of phase transition or of protein transconformation, acting in opposite ways at these extreme ranges. Germination may be limited by diffusion processess only from 29.3 to 35.2 °C. The synchronization of the germination of individual seeds is always limited by a minority of slower germinating seeds. The thermal communication between the seed growth effector and the exterior takes place by a temperature signal superimposed upon random thermal noise.

In addition, another report referring to the temperature factor only, but in a more ecological aspect, is the one on *Salvia columbariae* (Capon *et al.* 1978) with a widespread distribution over a considerable range of environments through southwest USA and Sonora, Mexico, in such a way that the species is composed of several ecotypes, each characterized by adaptability to a specific microclimate, particularly to prevailing seasonal temperatures corresponding to different life cycle stages. Populations appeared relatively homogeneous. New genotypes introduced by cross-breeding may be rapidly eliminated by the failure of ambient temperatures to break the seed dormancy. Summer temperature was probably the most important factor affecting afterripening. Seeds from mountain sites were viable for longer periods than seeds from desert sites and showed some positive response to cold dry storage.

Finally, the light-temperature co-factor, involving seed germination, was examined in *Salvia mellifera* in comparison with chaparral and desert scrub in Southern California (USA) (Keeley 1986). The seed failed to germinate unless

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exposed in powdered charred wood. This pattern was observed for seeds given an one month-long stratification at 5 °C as well as for seeds not stratified and seeds incubated under continuous 23 °C or a diurnal alteration of 13 °C/23 °C. Seeds from the coastal sage were germinated readily in light conditions. There were numerous significant interactions between incubation temperature, pre-chilling stratification, light and heating/charred wood treatments. Timing of germination was remarkably consistent between populations; the vast majority of seeds germinated within the first week at 23 °C (or altering 13/23 °C), regardless of whether or not they had received a pre-chilling treatment.

GROWTH COMPETITION AND NATURAL SELECTION

Effects of Density

In order to determine the response of planting density in *Salvia lyrata,* a perennial herb, Shaw (1986) took the progeny of a random collection of the herb and planted it into the source field in a range of densities and into several closely neighbouring locations. The highest density and particular locations inducted significantly greater mortality relative to the remaining densities and locations, indicating major effects of density and spatial location in fitness. Over the duration of the entire study, there was also significant variation among families in mortality. The survival data gave no indication of variation among families in their responses to the range of environments. Conversely, results based on growth and size indicated that different families were favored in different densities and locations, in support of the hypothesis that environment-dependent selection promotes specialization to different environments in this species. The correlation among families between leaf number in high and low density was small and positive, indicating near-independence of performance in different densities.

Another work of Shaw (1987) reports on the density-dependence of *Salvia lyrata,* in natural populations (established plants). Two experiments were conducted in order to investigate the effects of conspecific density on survival, fecundity and growth of established individuals. In both experiments, the density around monitored *Salvia* individuals was altered, either by removal of surrounding *Salvia* plants within a specific distance, or planting *Salvia* in as neighbours. Regarding survival, fecundity and growth, in both experiments, small responses to density manipula tions were observed. The responses varied with individual size; in large individuals these traits tended to decrease with increasing density, whereas small plants were in several aspects favoured by more crowded conditions. The weakness of the responses to neighbours suggests that the population of mature *Salvia* is not strongly self-regulating at typical natural densities or that marked effects of crowding appear only after a delay of more than two years. Mortality at the seedling stage as well as density-independent determinants of growth appear to maintain the adult popula tion below the threshold at which neighbour interactions have strong effects.

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Relative to seed densities, during a four-year long demographic report Shaw and Antonovics (1986) observed that the established number of seedlings of *Salvia lyrata* L. varied widely from year to year. The half-life of the seedling population was about 10 months and seedling mortality declined with time. Correlation of seedling mortality with population density was low. Also field experiments showed that established *S. lyrata* individuals significantly reduced the proportion of seedlings emerging and surviving. Seed density had no demonstrable effect of the proportion of seedlings emerging over a range of densities (0–1440 seeds/dm^2). There was thus no evidence of regulation by limitation of "safe sites" in this species in nature. Seedling mortality depended strongly on seed and seedling compared with densities of 240 and 480 seeds/dm^2. Seedling growth was negatively density dependent. Growth reduction suffered at initially high densities and persisted after a large fraction of individuals had died. Regulation numbers of *S. lyrata* seedlings had occurred primarily through the negative effects on adults.

Competition with other Species

Interactions between *Salvia reflexa* Hornem. and other species were reported by Weerakoon and Lovett (1986). The growth productivity was greater in the field where it competed with a summer crop (sorghum), than with a winter crop (wheat). In a glasshouse experiment, the growth of *S. reflexa,* with *Digitaria smutzii* Stent. and *Phalaris aquatica* L. *cv.* "Sirocco" was much modified by nutrient and defoliation treatments. *D. smutzii* was a strong competitor in warm growing conditions, even in drought and with frequent defoliation. With application of phosphorus and nitrogen, *D. smutzii* gain a competitive advantage over *S. reflexa.* When *S. reflexa* was grown with P. *aquatica* under these conditions, *S. reflexa* was more competitive than *P. aquatica,* especially at high nutrient levels. Stress (defoliation or drought) adversely affected *P. aquatica* growth and competitive ability. *S. reflexa* has a modest invasive capacity in pastures which, coupled with its tolerance to adverse environmental conditions, could cause significant weed problems in poorly managed pastures.

Finally, it has been reported (Lovett and Lynch 1979) that when grown in mix tures with equal numbers of wheat plants, *S. reflexa* was not a strong competitor. The height an inflorescence dry weight of wheat was increased by watering the mixtures from above the foliage. These effects were accentuated by moisture stress. Aqueous leachates of *S. reflexa* foliage inhibited the germination and/or early growth of wheat grown on filter paper in petri dishes and in different soil types.

Seed Color Adaptation

In a report (Brayton and Capon 1980) on variations in seed morpology among 19 populations of the annual *Salvia columbariae* Benth., it was noted that a high frequency of colour matching occurs between seeds and soil at individual population and sites. Seed and soil colours ranged from grey to light brown and red-brown. During an intervening 8-year period, the constancy of seed colour in 26 populations

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was verified. Seed colour was most uniform in relatively small populations (25–50 plants) that occupied sites with a single soil colour. In large populations (> 500 plants) that cover patchy areas of mixed soil types, seed colour was variable with no localized patterns of seed-soil colour matching. Close correspondence between seed and soil colour in small populations suggested that natural selection in favour of seeds least easily seen by granivores was operative. The results of field and laboratory experiments supported the selection hypothesis.

OTHERS

Mycorrhizal Associations

The development of a vesicular-arbuscular mycorrhizal association in *Salvia hispanica* L. was examined in comparison with light conditions (Muhammad and Hussain 1995). Roots of *S. hispanica* L. grown under light exhibited a gh percentage (87.0%) and an rage intensity (29.42%) of mycorrhizal infection than those growing in partial light conditions. The percentage (71.0%) and average intensity (23.07%) of abuscular infection was high in full light-grown plants. Extramatricial vesicles, intramatricial hyphal coils, beaded mycelium and multiple vesicular-arbuscular mycorrhizal (VAM) associations were the characteristic features of infection.

Herbicide Tolerance

Experiments involving the Syrian sage *(Salvia syriaca)* were conducted in order to determine the possible chemical control of the plant (Qasem and Abu-Irmaileh 1983). Two experiments were done by spraying 2,4-D ester or MCPA (2-methyl-4-chlorophenoxy acetic acid) at 1 kg a.i. (active ingredient)/ha and their combination with dicamba (3, 6-dichloro-o-anisic acid) at 0.5+0.15 kg a.i./ha and 1+0.15 kg a.i./ha (two different locations) applied at the pre-flowering stage of the weed. Syrian sage competed with wheat and significantly reduced yield. Removing the weed 2 or 4 weeks after its emergence improved the grain and straw yield significantly at both locations. Spraying MCPA at 1 kg a.i./ha and combination of MCPA and dicamba (1 kg a.i. MCPA+0.15 kg a.i. dicamba/ha) resulted in good control of the weed. The treatments increased significantly wheat yield over the treatments. 2,d-D at 1 kg a.i./ha resulted in good control of Syrian sage but its effect was limited in time and magnitude.

Allelopathic Interactions

Scanning electron microscope examination of adaxial leaf surfaces of *Salvia reflexa* revealed specialized structures from which chemical release was apparently promoted by immersion in water. The significance of these structures and their contents has been discussed in relation to the allelopathic activity of this species (Lovett and Speak 1979).

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10. LEGAL PROTECTION OF *SALVIA* VARIETIES

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INTRODUCTION

Contrary to varieties of agricultural and horticultural species which, in several countries, must be registered in an official national catalogue before being granted authorization for commercialization, varieties of ornamental, aromatic and medicinal species can be commercialized without prior registration. Thus, there is no official catalogue for varieties of these species.

This is probably due to the fact that, in most of the cases, the varieties used are non- or little improved local populations.

The *Salvia* genus does not escape this rule. Nevertheless, as previously shown in Chapter 8, breeding work is currently done in some countries to obtain varieties with improved characteristics, in their agricultural behaviour as well as in their chemical composition. Breeders would like to be granted intellectual property rights allowing them to improve their return on investment. This wish is justified because breeding new varieties of plants requires substantial investment in terms of skill, labour, material resources and money and may take years. In addition, a new variety, once released, can in many cases be readily reproduced by others so as to deprive its breeder of the opportunity to profit adequately from his investment. Granting a breeder of a new variety the exclusive right to replant or to license his variety, for a limited period of time, both encourages him to invest in plant breeding and contributes to the development of correlated industries.

CURRENT SITUATION OF PROTECTION

A quick search on patents, in a data bank, in October 1997, showed that currently there are 819 patents referring to the genus *Salvia* or to its English equivalent Sage. When limiting the search to the precise Latin key word *Salvia* there are 227 references remaining and, if concentrating on *Salvia officinalis*, there are 44 references left. However, a quick analysis of the latter shows that in fact not the varieties are protected but the utilization of some components extracted from the plant for aromatic, cosmetic or medicinal purposes.

On the other hand, the analysis of the UPOV data bank, also in October 1997, showed that only 3 applications for protection of *Salvia* varieties have been lodged,

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one in Israel, the second one in Japan and the third one in the United States and that, for the time being, only the American title has been granted. These three applications are concerning ornamental varieties.

Whereas patent protection of the utilization of compounds extracted from sage varieties is a routine activity, the protection of the variety itself is exceptional for ornamental varieties and inexistent for medicinal and aromatic varieties.

HOW TO PROTECT PLANT VARIETIES

Apart from three countries, Japan, Australia and the United States, the protection of plant varieties by industrial patents is generally legally not accepted. Practically, this possibility is frequently used only in the United States, and some wonder about the validity of these patents, particularly in view of the patentability criterion of non-evidence in the American law or, more generally, of inventive step in international conventions. There has been no jurisprudence on this subject so far.

The plant protection system most frequently used in the world is the system of UPOV, Union pour la protection des obtentions vegetales (Union for the Protection of New Varieties of Plants), created in 1961. Currently, the 44 following countries are members of UPOV: Argentina, Austria, Australia, Belgium, Bolivia, Brazil, Bulgaria, Canada, Chile, China, Colombia, Czech Republic, Denmark, Ecuador, Finland, France, Germany, Hungary, Ireland, Israel, Italy, Japan, Mexico, Moldavia, Netherlands, New Zealand, Norway, Panama, Paraguay, Poland, Portugal, Russian Federation, Slovakia, Slovenia, South Africa, Spain, Sweden, Switzerland, Trinidad and Tobago, Ukraine, United Kingdom, United States, Uruguay.

Furthermore, the GATT Agreement signed in Marakesh in 1994 states that all WTO member countries shall protect plant varieties either by patent, or by a sui generis protection system or by a combination of both. Many member countries are likely to adopt either the UPOV system or a similar one.

THE UPOV SYSTEM

The first Act of the UPOV Convention was signed in 1961 and revised in 1972, 1978 and 1991. The 1991 Act of the Convention entered into force in 1998 after ratification by 5 countries. The 1978 Act is no longer accessible. So we will focus in this chapter on the 1991 Act of the Convention.

Main Dispositions of the 1991 Act of the UPOV Convention

Definition of a variety

Variety means a plant grouping within a single botanical taxon of the lowest known rank, which grouping, irrespective of whether the conditions for the grant of a breeder's right are fully met, can be

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- defined by the expression of the characteristics resulting from a given genotype or combination of genotypes,
- distinguished from any other plant grouping by the expression of at least one of the said characteristics and
- considered as a unit with regard to its suitability for being propagated unchanged.

It is of course important for a plant breeder to know that definition prior to lodging an application for plant protection, as it is used for defining the conditions for protection.

Protection of all genera and species

In the former Acts of the UPOV Convention, the protection of plant varieties of all genera and species, whilst encouraged, was not compulsory. The minimum number of protected genera and species is, in the 1978 Act, 24 within 8 years following the date of entry into force of the Convention in a given country.

The 1991 Act provides for the protection of all genera and species within 5 years following the date of entry into force of the 1991 Act in countries bound by the Acts of 1991/1972 or the Act of 1978 and within 10 years for the other countries.

This new provision is, of course, favourable to the varieties of medicinals and aromatics as, up to now, they were not considered as having priority by the plant protection offices.

Conditions for the grant of the breeder's rights

The UPOV Convention states that the breeder's right shall be granted where the variety is

- new,
- distinct,
- uniform and
- stable
- designated by a denomination in accordance with the provisions of the Convention.

The grant of the breeder's right shall not be subject to any further or different technical conditions.

Novelty

The variety shall be deemed to be new if, at the date of filing of the application for a breeder's right, propagating or harvested material of the variety has not been sold or otherwise disposed of to others, by or with the consent of the breeder, for purposes of exploitation of the variety.

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i) in the territory of the Contracting Party in which the application has been filed earlier than one year before that date and
ii) in a territory other than that of the Contracting Party in which the application has been filed earlier than four years or, in the case of trees or of vines, earlier than six years before the said date.

It is interesting to note that where a country applies the UPOV Convention to a plant genus or species which it did not previously grant protection, it may consider a variety of recent creation existing at the date of such extension of protection as new even where the sale or disposal to others described above took place earlier than the time limits defined in that paragraph. As it will be the case for *Salvia* in most of the countries, it will give more possibilities to plant breeders to protect their varieties.

Distinctness

The variety shall be deemed to be distinct if it is clearly distinguishable from any other variety whose existence is a matter of common knowledge at the time of the filing of the application. In particular, the filing of an application for the granting of a breeder's right or for the entering of another variety in an official register of varieties, in any country, shall be deemed to render that other variety a matter of common knowledge from the date of the application, provided that the application leads to the granting of a breeder's right or to the entering of the said other variety in the official register of varieties, as the case may be.

Uniformity

The variety shall be deemed to be uniform if, subject to the variation that may be expected from the particular features of its propagation, it is sufficiently uniform in its relevant characteristics.

Stability

The variety shall be deemed to be stable if its relevant characteristics remain unchanged after repeated propagation or, in the case of a particular cycle of propagation, at the end of each such cycle.

In the quasi totality of UPOV member states, distinctness, homogeneity and stability tests are effected according to test guidelines published by the office of the Union. For the time being, there are no guidelines for the genus *Salvia* and it would be interesting that breeders of this genus publish a widely accepted botanical description which could serve as a basis for UPOV guidelines. For the moment, there are only very few guidelines for medicinal and aromatic plants: camomile, ginger, gentian, parsley, garlic, Welsh onion, juniper.

The rights of the breeder

Where a breeder has been granted a right on a variety, the following acts in respect of the propagating material of the protected variety shall require his authorization:

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i) production or reproduction (multiplication),
ii) conditioning for the purpose of propagation,
iii) offering for sale,
iv) selling or other marketing,
v) exporting,
vi) importing,
vii) stocking for any of the purposes mentioned in (i) to (vi), above.

The breeder may subject authorization to conditions and limitations.

When a breeder has had no reasonable opportunity to exercise his right in relation to the propagating material, his authorization is required for production or reproduction, conditioning for the purpose of propagation, offering for sale, selling or other marketing, exporting, importing, stocking of harvested material, including entire plants and parts of plants obtained through the unauthorized use of propagating material of the protected variety.

The rights regarding the propagating material and the harvested material of a protected variety, as described above, are compulsory and must be granted if the variety fulfills the criteria for protection, by all UPOV member states.

A new optional provision, subject to the decision of the UPOV member states, regarding the products made directly from harvested material of the variety, is included in the 1991 Act of the UPOV Convention: when a breeder has had no reasonable opportunity to exercise his right in relation to the propagating material or the harvested material of the protected variety, his authorization is required for the use of products made directly from the harvested material of the protected variety obtained through the unauthorized use of the material of the variety.

That new provision is of particular interest to the breeders of medicinal and aromatic plants when it is possible to characterize a particular compound produced by a protected variety. However, it must be noted that most of the revised national laws, consistent with the 1991 Act of the UPOV Convention, do not include that voluntary provision. Breeders of medicinal and aromatic plants should try to convince their governments of the interest of such a clause.

Exception to the breeder's right

The UPOV Convention, since 1961, has always provided for compulsory exceptions to the breeder's right related to

- acts done privately and for non-commercial purposes,
- acts done for experimental purposes,
- acts done for the purpose of breeding other varieties and, except where the new variety is essentially derived from an initial protected variety, acts presented above within the scope of breeder's right in respect of such other varieties.

That latter exception is generally known as breeder's exception.

In the 1991 Act of the UPOV Convention, an optional exception was introduced related to farm-saved seed: each Contracting Party may, within reasonable limits and

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subject to the safeguarding of the legitimate interests of the breeder, restrict the breeder's right in relation to any protected variety in order to permit farmers to use for propagating purposes, on their own holdings, the product of the harvest which they have obtained by planting, on their own holdings, the protected variety.

The breeder's exception is considered essential by the members of the International Association of Plant Breeders, ASSINSEL, and has to be maintained in the UPOV Convention and introduced into any sui generis system required by the TRIP'S Agreement. However, the breeder's exception can, sometimes, lead to piracy and/or plagiarism. It is to avoid such piracy/plagiarism that the new concepts of essential derivation and dependence have been introduced into the 1991 Act of the UPOV Convention.

Essentially derived variety

According to the UPOV Convention, a variety is deemed to be essentially derived from another variety, called initial variety, when

- it is predominantly derived from the initial variety, or from a variety that is itself predominantly derived from the initial variety, while retaining the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety;
- it is clearly distinguishable from the initial variety and
- except for the differences which result from the act of derivation, it conforms to the initial variety in the expression of the essential characteristics that result from the genotype or combination of genotypes of the initial variety.

For example, an essentially derived variety may be obtained by the selection of a natural or induced mutant, or of a somaclonal variant, the selection of a variant individual from plants of the initial variety, backcrossing or transformation by genetic engineering.

If a variety is essentially derived from an initial protected variety, the breeder who has been granted the right regarding the essentially derived variety must have the authorization of the owner of the protected initial variety (dependence) before commercializing the essentially derived variety.

CONCLUSION

The 1991 Act of the UPOV Convention and the 1994 TRIP's Agreement provide for the possibility of protecting all genera and species in many countries. Hence, this gives a new opportunity to breeders of ornamental, medicinal and aromatic plants and in particular to *Salvia* breeders.

In order to initiate the protection of species of *Salvia* genus and to let this new system get into its stride, breeders in countries, which provide now for this possibility, should

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- apply for protection,
- establish widely accepted botanical descriptions which could serve as a basis for UPOV Test Guidelines.

It would also be important that breeders of aromatic and medicinal plants obtain from their governments that the scope of protection be extended to the harvested material of the protected variety and to the products made directly from the harvested material of the protected variety.

The legal instruments, even though they are still imperfect, exist and it is up to the breeders to make use of them so as to try and then improve them if need be. An efficient protection of the intellectual property should enhance the breeding work on the *Salvia* genus.

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V. PHARMACOLOGY
11. THE BIOLOGICAL/PHARMACOLOGICAL ACTIVITY OF THE *SALVIA* GENUS

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INTRODUCTION

Sage (*Salvia* species) has been used as a herb with beneficial healing properties for millennia. The name itself comes from the Latin word for health (*salvare* or heal). Ancient authors called it *elelisphakon*. This term most likely refers to several species, such as *Salvia fruticosa* Mill., *Salvia officinalis L. and Salvia pomifera* L. (Rivera *et al.,* 1994). A tenth century Salerno School called it *Salvia salviatrix,* whereas the Spanish call it *ierba buena* or "good herb". Both terms admire feats attributed to sage. A proverb assures us, that a man who has sage in his garden needs no doctor. Sage became very popular also in China in the eighteenth century where the merchants would exchange two crates of best tea for a crate of sage (Toussaint-Samat, 1996).

Until the discovery of antibiotics, sage was a frequent component of herbal tea mixtures, recommended in patients with tuberculosis to prevent sudation. The essential oil of sage is still employed in flavouring condiments, cured meats, liqueurs and bitters. Besides the usage as a flavouring and antioxidant agent, sage (*S. officinalis* L.) leaves exhibit a range of biological activities, i.e. antibacterial, micostatic, virustatic, astringent and antihidrotic (Anonymus, 1994). Sage was found to be an active ingredient in combined plant preparations for treatment of acute and chronic bronchitis. Animal studies show hypotensive activity and central nervous system (CNS) depressant action of sage extracts (Newall *et al., 1996*). Because of antimicrobial effects (Dobrynin *et al.,* 1976; Cherevatyi *et al.,* 1980; Farag *et al.,* 1986) and tannin-based astringent activities of sage this is used as an active ingredient of dental-care preparations. It reduces growth of plaques, inhibits gingival inflammation, and has beneficial effects on caries prophylaxis (Willershausen *et al.,* 1991).

The traditional Chinese herbal drug Dan-Shen (Tan-shen, *S. miltiorrkiza* Bge.) is described to have sedative, antimicrobial, antispasmodic, anti-inflammatory and antioxidant properties. Tan-shen is mentioned in Chinese Pharmacopoeia as a drug that treats problems associated with heart and circulatory system (oral preparations: decoction or tablet with *Panax notoginseng*) insomnia (dry, oral preparation: decoction with *Polygala tenuifolia* and *Zizyphus spinosa*) and as a drug used in the

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treatment of acute arthritic pain in patients with rheumatism (Xiao, 1989). Also, use of the decoction of tan-shen together with other herbs such as *Angelica sinensis* and *Curcuma zedoaria,* is recommended in the treatment of amenorrhea, dysmenorrhea and other menstrual disorders. When studying the effects of *S. miltiorrhiza* on endocrine function of ovary-uterus in immature rats, increased level of estradiol (E2) in plasma, weight of uterus and ovarian PGF2 alpha content were observed by Li *et al.* (1992). *S. Miltiorrhiza* stimulated ovulation in immature mice, inhibited function of corpus luteum in pseudopregnant rats and decreased concentration of progesterone in plasma.

S. haematodes Wall., known as red sage, was found to posses significant CNS depressant (anticonvulsant) properties (Akbar *et al.,* 1985). Further pharmacological screening revealed a broad variety of pharmacological effects. When tested in animal models, the ethanolic extract of red sage showed anti-inflammatory and analgesic effects, hypothermic response in non-pyretic rats and enhancement of the wound healing process (Akbar, 1989). The ethanolic extract of *S. haematodes* had significant inotropic and chronotropic effects on isolated rabbit hearts. It also had a parasympathomimetic effect on isolated rabbit duodenum. Unfortunately, active substances responsible for these effects have been so far unknown, although different constituents are probably involved.

S. desoleana Atzei & Picci, an indigenous Sardinian species, is used in folk medicine to treat menstrual and digestive disorders and diseases of the central nervous system. Peana and Satta (1992) reported that the essential oil from the leaves of *S. desoleana* had a dose dependent central nervous-depressant effect in mice. In further pharmacological screening, the essential oil was tested also for its choleretic effects in rats and was found to significantly increase bile flux at 1 h after administration of essential oil at 250 mg/kg *i.p.* or its alcoholic (linalool and alpha-terpineol) fraction at 62 mg/kg i.p. (Peana *et al.,* 1994). Intraperitoneal administration of essential oil produced stronger choleretic effects than subcutaneous administration. The amount of dry bile residue of essential oil treated rats was higher than that of control values at 1 and at 2 h after treatment. Linalool and alpha-terpineol fractions of the essential oil fractions showed the strongest choleretic activity.

A wide variety of species (900 known species) of the *Salvia* genus shows also much variety in bioactivity. There are, however, many differences in pharmacol ogical effects amongst these species. Aerial parts of these plants usually contain flavonoids and triterpenoids as well as essential oils with volatile compounds such as monoterpenoids. Diterpenoids are the main compounds in roots. Some of these compounds have been isolated and their structures elucidated, however, many compounds are still scientifically challenging. Many more studies on structure-activity relationship within the *Salvia* species are needed in order to explain mechanisms of biological activity.

ANTIMICROBIAL AND ANTIVIRAL ACTIVITIES

Extensive literature on the antimicrobial potency of the *Salvia* genus reveals a broad variability with regard to microorganisms sensitivity as well as to the efficiency

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(measured as minimal inhibitory concentration, MIC) of tested compounds, when different species are considered. Most frequently, essential oils with volatile monoterpenoids as their major constituents are reported to be antibacterially active in those *Salvia* species that are rich in essential oil (*S. officinalis* L., *S. lavandulifolia* Vahl., *S. triloba* L.=*S. fruticosa* Mill.).

Less evidence exists on the antifungal potency of these essential oils. Generally, Gram-negative bacteria are not sensitive or are less sensitive for sage essential oil when compared with the sensitivity of Gram-positive bacteria. This is in agreement with observations of Maruzella and Henry (1958) and of Yousef and Tawil (1980), while some authors report that there is no relationship between susceptibility of tested bacteria to essential oils and their Gram reaction (Deans and Ritchie, 1987, Shapiro *et al*., 1994).

When compared with some other species from *Labiatae* family (especially *Thymus* spp. and *Origanum* spp.), essential oils of *Salvia* species show relatively low antibacterial and/or antifungal activity (Thompson *et al.,* 1986).

Sage oil turned out to exhibit inhibitory effects on many of oral bacteria, such as obligate anaerobes *(Fusobacterium nucleatum, Peptostreptococcus anaerobius, Porphyromonas gingivalis, Treponema denticola, Treponema vincentii)* and capnophilic microaerophiles (*Actinobacillus actinomycetemcomitans, Capnocytophaga* spp., *Eikenella corrodens*) at concentrations between 0.06 % (w/v) and 0.2 % (w/v). When compared to obligate anaerobes, the facultative anaerobe group of oral bacteria *(Actinomyces viscosus, Strepococcus sanguis, Streptococcus sobrinus)* was generally less sensitive to administered sage oil. Sage oil inhibited the growth of the facultative group at concentrations between 0.3% (w/v) and 0.6 % (w/v) (Shapiro *et al.,* 1994).

The Egyptian sage essential oil, composed mostly of thujone (41.5%) and of limonene (14.7%), shows antibacterial activity against Gram-positive *Sarcina* spp. (MIC=2.0 mg/ml), *Staphylococcus aureus* (MIC =1.0 mg/ml), *Bacillus subtilis* (MIC=0.75 mg/ml) and against yeast *Saccharomyces cerevisiae* (MIC 2.0 mg/1) (Farag *et al.,* 1989a). According to Kustrak and Pepeljnjak (1989), the antimicrobial activity (against *Bacillus subtilis*) of sage oil depended on composition, i.e. contents of 1, 8-cineole, p-cymene, *a*- and *ß*-thujone and camphor as well as on the relationship between 1, 8-cineole, p-cymene and ketonic compounds. The antimicrobial activity of Dalmatian sage oil, was attributed to its thujone contents (Jalsenjak *et al*., 1987). Antibacterial activity was not reduced even when essential oil was microencapsulated into gelatin-acacia capsules (although a certain time lag in achieving full activity was observed), microencapsulation, however, inhibited antifungal activity of sage oil.

According to Deans and Ritchie (1987), who tested 50 essential oils against 25 genera of bacteria, sage (*S. officinalis* L.) essential oil (undiluted) was moderately effective against the growth of *Bacillus subtilis, Brevibacterium linens, Micrococcus luteus, Seratia marcescens* bacteria. When tested against eight bacteria *(Bacillus subtilis, Escherichia coli, Hafnia alvei, Micrococcus luteus, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus faecalis)* and five fungi (*Aspergillus niger, Aspergillus terreus,* two strains of *Candida albicans,*

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Fusarium spp.) commercial sage essential oil (probably issued from a mixture of *S. triloba* L. and of *S. lavandulifolia* Vahl.) had almost no effect (Biondi *et al.,* 1993). Similarly, the essential oil from *S. triloba* showed no fungistatic activity against soil-borne pathogens *(Fusarium oxysporum, Macrophomina phaseolina)* or against foliar plant pathogens *(Botrytis cinerea, Exserohilum turcicum)* (Shimoni *et al.,* 1993). Ground sage (2%), as a component of Malt Extract Agar (MEA) medium, showed no fungistatic activity against food-contaminating fungi *(Trichoderma harzianum, Alternaria alternata, Fusarium oxysporum, Mucor circinelloides f. griseo-cyanus, Rhizopus stolonifer, Cladosporium cladosporioides, Fusarium culmorum, Aspergillus versi-color, Penicillium citrinum)* (Schmitz *et al.,* 1993).

Contrary to this, by measuring the antifungal property of sage essential oil against *Alternaria alternata* and against *Aspergillus parasiticus,* a strong fungistatic effect was observed (Crisan and Hodisan, 1975; Farag *et al.,* 1989b). Volatile oils showed much stronger fungistatic properties than tested extracts (Crisan and Hodisan, 1975).

Concentration of 2.0 mg/ml sage oil reduced *Aspergillus parasiticus* mould growth by 87.6% and inhibited total aflatoxin (B and G groups) production by more than 96% (Farag *et al.,* 1989b). Like antibacterial activity, the mould growth inhibitory effect of sage oil was mainly due to thujone as a major component in essential oil. According to Farag *et al.* (1989a, 1989b), a relationship between the chemical structures of the most prevalent compounds in essential oils and antimicrobial activity was observed. The antimicrobial activity of sage essential oil was lower than that of essential oils (thyme oil, clove oil) containing thymol or other phenolic-OH structure compounds (eugenol). A well known inductive effect of polar functional groups (e.g. hydroxyl or isopropyl) on aromatic nucleus seems of great importance in explaining the correlation between structure and antimicrobial activity of the essential oil compound.

The essential oil of *S. plebeia* is also reported to have fungitoxic potential, inhibiting the growth of storage fungus *Aspergillus flavus* by 54% (at a concentration of 5000 ppm) (Mishra and Dubey, 1990).

Much emphasis has also been placed on the investigation of compounds (i.e. diterpenoids, flavonoids) in extracts of different *Salvia* species, which show significant inhibitory activity against bacteria (G-negative and/or G-positive) and fungi.

Significant antibacterial (towards gram-negative *Klebsiella pneumoniae* at a concentration 400 µg/ml and against gram-positive *Bacillus subtilis* at 300 µg/ml and *Staphylocoecus aureus* (200 µg/ml) and antifungal (towards *Candida albicans* at concentration of 200 µg/ml) compounds (carnosic acid, 16-hydroxycarnosic acid and their derivatives) were found in the diterpene acid fraction of extract of *S. apiana,* whereas its essential oil (composed primarily of 1, 8-cineole and camphor) as well as a mixture of oleanolic and ursolic acid were inactive even at 1000 µg/ml against tested organisms (Dentali and Hoffmann, 1992). Antibacterial activity (against *Staphylococcus aureus*) of carnosic acid (referred to as salvin) has been reported already by Dobrynin *et al.* (1976) and Pavlenko *et al.* (1989). The dry methanolic extract of *S. officinalis,* dissolved in DMSO (50 mg/ml DMSO) inhibited the growth of Gram-positive *Staphylococcus aureus* at a concentration 100 µg/ml, while no

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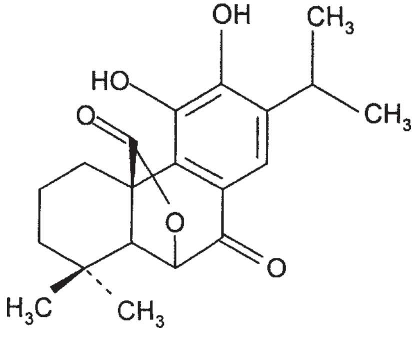
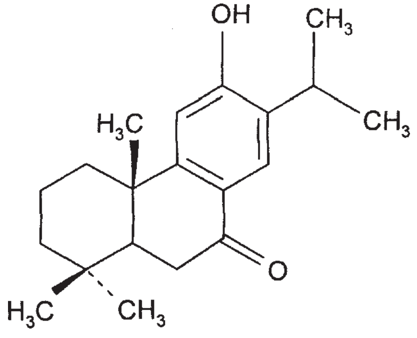


Structure 1

antibacterial activity against Gram-negative bacteria *E. coli* or *Pseudomonas aeruginosa* strains was observed (Baricevic *et al.,* 1996). An abietane diterpene galdosol *(structure 1)* with antibacterial properties against *Bacillus subtilis, Micrococcus luteus and Staphylococcus aureus* was isolated from the aerial parts of *S. canariensis* L., a plant endemic to Canary Islands (Gonzalez *et al.,* 1989a; Gonzalez *et al.,* 1989b; Darias *et al.,* 1990). This shrub has been used in folk medicine as an antispasmodic, febrifuge and hypoglycemiant. Abietane diterpenes, sugiol *(structure 2)* and 15-hydroxy-7-oxo-abiet-8,11,13-triene *(structure 3),* were isolated from S. *albocaerulea* Lindl., a species, which is native to the south-eastern intertropical region of Mexico. These two diterpenes are responsible for antimicrobial activity against Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus,* MIC 50 µg/ml) and for a moderate activity against *Candida albicans* (at 100 µg/ml), but are inactive against Gram-negative bacteria (Pereda-Miranda *et al.,* 1992). Another abietane diterpene, forskalinone *(structure 4)* isolated from roots of *S. forskahlei* L., showed slight antimicrobial activity against *Enterococcus faecalis* (168 µg/ml) (Ulubelen *et al.,* 1996). Free catechol grouping (or



Structure 2.

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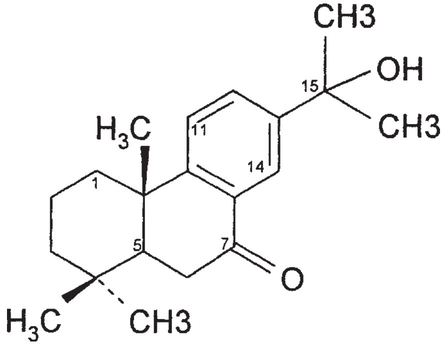
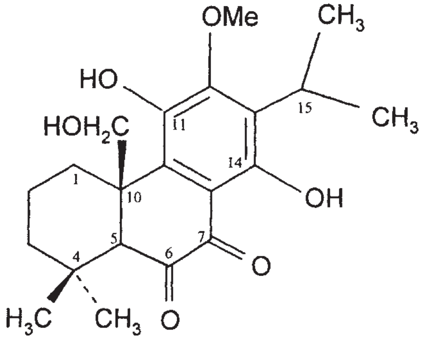
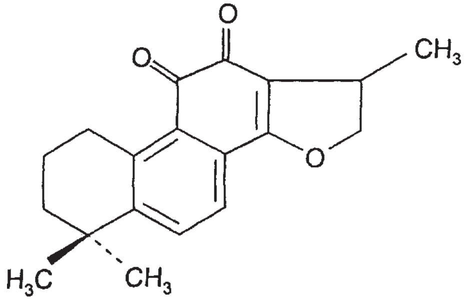


Structure 3



Structure 4



Structure 5

its oxidised quinone structure) is responsible for the antimicrobial activity of *Salvia* abietane diterpenes against Gram-positive bacteria (Moujir *et al.*, 1993).

A series of phenanthrene quinone derivatives has been identified in S. *miltiorrhiza* (Kakisawa *et al.*, 1968; Kakisawa *et al.*, 1969; Shibata *et al.*, 1982; Xiao and Fu,

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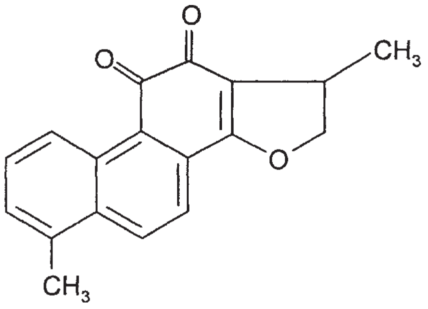
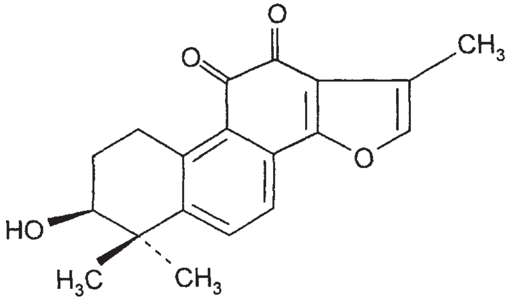
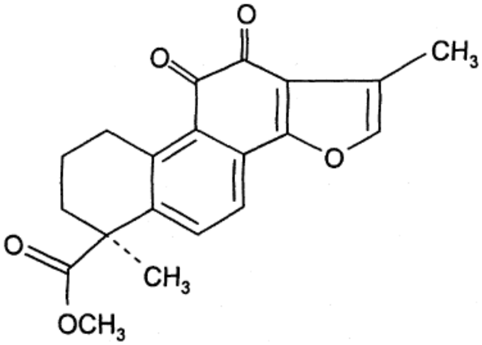
1987). The medicinal mixture known as tanshinone, as well as components of mixture such as cryptotanshinone *(structure 5),* dihydrotanshinone I *(structure 6),* hydroxytanshinone II-A *(structure 7),* methyltanshionate *(structure 8)* and tanshinone II-B *(structure 9),* were shown to have bacteriostatic activity, especially on



Structure 6



Structure 7



Structure 8

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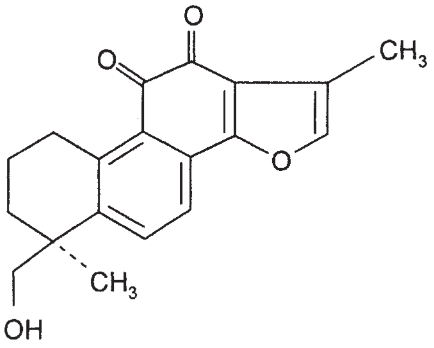
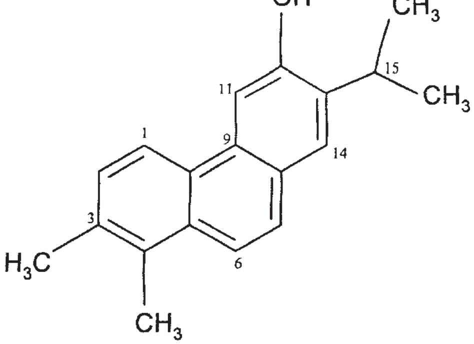


Structure 9

Staphylococcus aureus strains cultured *in vitro*. Tanshinones didn't show any antimicrobial activity against gram-negative bacteria *(E. coli, Pseudomonas aeruginosa, Citrobacter freundii, Serratia marcescens)* and yeasts *(Candida albicans, C. krusei, C. mycoderma, C. tropicalis, C. utilis, Saccharomyces sake)* at a concentration of 100 µg/ml (Honda *et al.*, 1988). While having a similar inhibitory effect against gram-positive bacteria (MIC 0.195–50 µg/ml), dihydrotanshinone I and cryptotanshinone differ fundamentally in their activity against dermatophytes. Dihydrotanshinone I (but not cryptotanshinone) was proved to be a potent antidermatophytic substance. This compound inhibited the mycelial growth of six dermatophytes (*Trichophyton rubrum, T. mentagrophytes, T. tonsulans* var. *sulfureum, Mycrosporum gypseum, Sabourandites canis, Epidermophyton floccosum*) at a concentration as low as 1.56 to 6.25 µg/ml, an activity being comparable to that of griseofulvin (Honda *et al.*, 1988). Tanshinone demonstrated inhibitory activity against *Mycobacterium tuberculosis* H 37 Rv and two related dermatophytes. Tablets and ointment of tanshinone provided satisfactory clinical results in 455 cases of infection mainly with *Staphylococcus aureus* (Gao *et al.*, 1979; Xiao and Fu, 1987). A significant antituberculous activity, tested on *Mycobacterium tuberculosis* H 37 Rv, was showed



Structure 10

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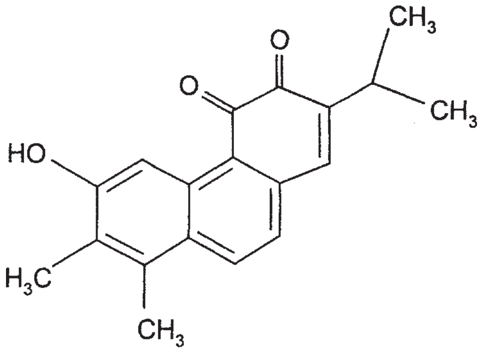
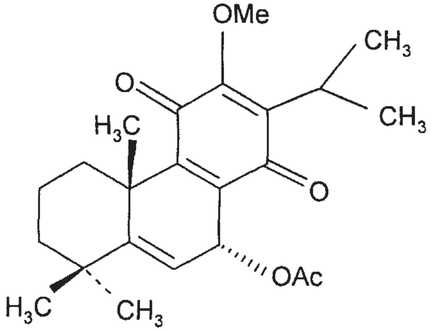


Structure 11



Structure 12

also by norditer-penoids and diterpenoids from *S. multicaulis,* the most potent substances being 12-demethylmulticaulin *(structure 10),* 12-demethylmultiorthoquinone *(structure 11)* and 12-methyl-5-dehydroacetylhorminone *(structure 12)* (Ulubelen *et al.,* 1997). Among anitibacterial active hypargenins, i.e. abietane diterpenoids isolated from the root extract of *S. hypargeia,* hypargenin F *(structure 13)* showed antituberculous activity (Ulubelen *et al.,* 1988).

Also some flavonoids proved to be active against Gram positive and/or Gram negative bacteria. Cirsimaritin, a flavonoid isolated from the leaves of *S. palaestina* Bentham, showed a high activity against standard strains of *Staphylococcus aureus* (MIC=31.25 µg/ml; minimum bactericidal concentration, MBC=125 µg/ml), *Staphylococcus epidermidis* (MIC=62.5 µg/ml ; MBC=125 µg/ml), *E. coli* (MIC=45 µg/ml ; MBC=90 g/ml), *Pseudomonas aeruginosa* (MIC=31.25 µg/ml; MBC=125 µg/ml), *Proteus vulgaris* (MIC=31.25 µg/ml ; MBC=125 µg/ml) and *Klebsiella pneumoniae* (MIC=45 µg/ml ; MBC=90 µg/mJ) (Miski *et al.,* 1983).

A potent antiviral activity of crude extracts of sage *(S. officinalis)* is displayed by two abietane diterpenoids, which were isolated from sage aerial parts and their structure elucidated. Safficinolide *(structure 14)* was active against VSV (vesicular stomatitis virus), while sageone *(structure 15)* showed virus inactivation activity

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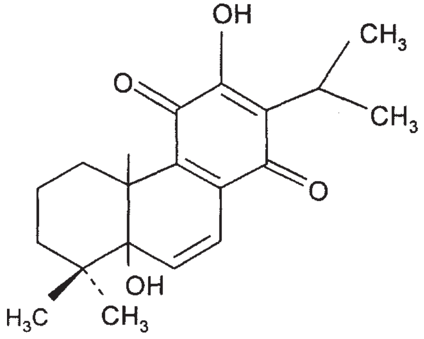
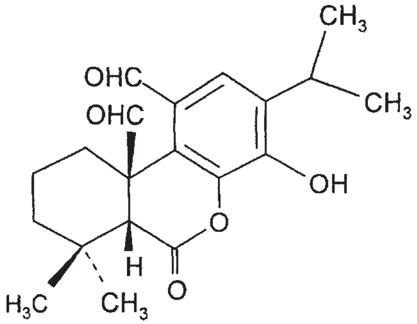
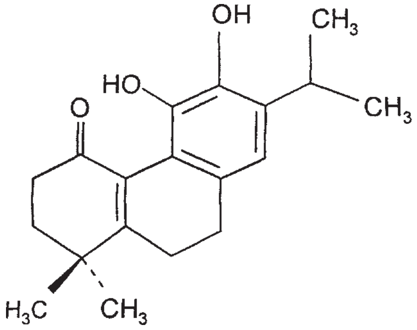


Structure 13



Structure 14



Structure 15

against HSV (herpes simplex virus type 1) (Tada *et al.*, 1994). Also, Sivropoulou *et al.* (1997) report on the high virucidal activity of essential oil of *S. triloba* against HSV. According to Bulgarian researchers, water and alcoholic extracts of sage were active against influenza, herpes simplex and vaccinia viruses (Manolova *et al.*, 1995). This preparation was officially approved for clinical use in Bulgaria.

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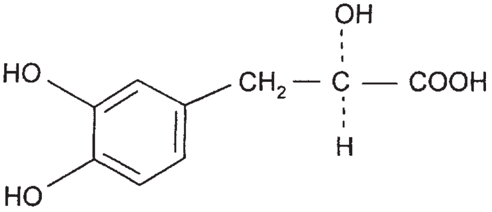
CARDIOVASCULAR AND RENAL ACTIVITIES

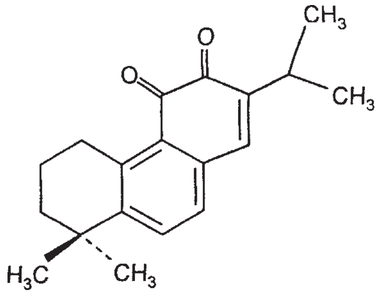
In China, herbal remedies made from *S. miltiorrhiza* roots are used by modern medicine to treat diseases such as cardio-cerebral ischemia, thrombosis, in the treatment of neurasthenic insomnia and in the prevention of myocardial infarction because they are capable of reducing aggregation of blood platelets, mobilising blood circulation, and of removing stasis (Chen, 1984; Chang and But, 1986; Lee *et al.,* 1987; Liu *et al.,* 1992). Danshensu, ß (3, 4-dihydroxyphenyl)-lactic acid *(structure 16),* obtained from the water-soluble fraction of *S. miltiorrhiza,* is reported to dilate coronary artery and significantly antagonise constricting responses elicited by morphine and propranolol (Dong and Jiang, 1982; Xiao and Fu, 1987).

The roots of *S. miltiorrhiza* were proved to inhibit cellular cholesterol biosynthesis (Sun and Cai, 1989) and to have vasodilatory, hypotensive, and anticoagulant properties. They are beneficial to patients with chronic renal failure (Chung *et al.,* 1986; Yokozawa *et al.,* 1990). However, use of the decoction of *S. Miltiorrhizae* in hypertension is questionable because it induces both vasodilatation and vaso-constriction what depends on the dosage and the target vessel (Lei and Chiou, 1986a; Lei and Chiou, 1986b). *S. Miltiorrhiza* dilated coronary vessels both at lower (3 mg/ml) or higher (10 mg/ml) concentrations, however, it contracted renal, femoral and mesenteric arteries at higher concentration (10.0 mg/ml) only.

Some sources identify phenolic compounds as the main source of a wide range of pharmacological properties. Among stasis-eliminating compounds abietane diterpenoids (miltirone—*structure 17,* Ro09–0680—*structure 18* and salvinone—



Structure 16



Structure 17

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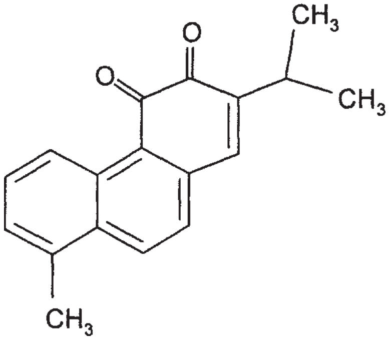
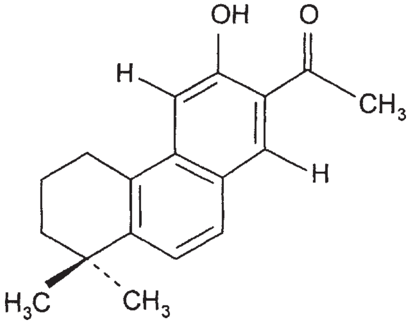


Structure 18



Structure 19

structure 19) showed a dose-dependent *in vivo* inhibiting properties in platelet aggregation in rabbits induced by collagen (Wang *et al.*, 1989). Yu and Xu (1994) report on the significant *in vitro* and *in vivo* inhibitory effect of acetylsalvianolic acid A (ASAA) on rat and rabbit platelet aggregation, induced by ADP, collagen and arachidonic acid. While inhibiting platelet aggregation, ASAA was found also to have a suppressive effect on collagen-induced 5-HT release. Zou *et al.* (1993) report on the antithrombotic effect of rosmarinic acid, which might be associated with its inhibition of platelet aggregation and promotion of fibrinolytic activity.

From the water soluble fraction of *S. miltiorrhiza,* an active principle, danshensuan B *(structure 20)*, a compound refered as lithospermic acid B by Tanaka *et al.* (1989), was isolated. This compound promotes fibrinolysis and increases coronary blood flow (Xiao and Fu, 1987). A potent inhibitory effect of compound IH764–3, isolated from *S. miltiorrhiza,* on fibroblast proliferation and on their ability to synthesise collagen, was reported by Liu *et al.* (1992).

S. Miltiorrhiza aqueous extract (decoction) was mentioned as a useful anti-anginal agent as it dilates coronary vessels (Lei and Chiou, 1986a; Lei and Chiou, 1986b). In isolated whole-heart preparations *S. Miltiorrhiza* it significantly

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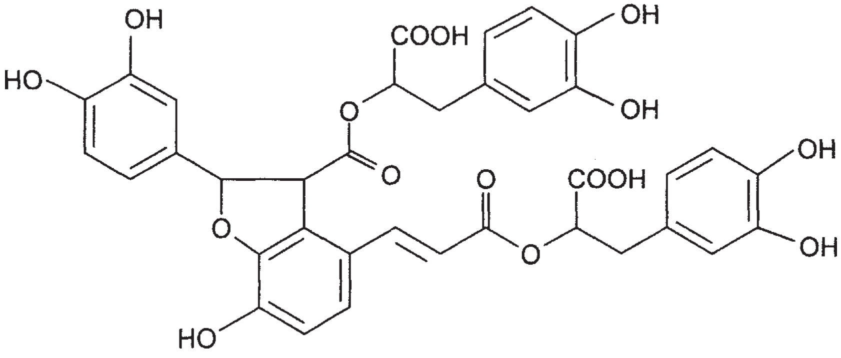

Structure 20

increased coronary blood flow for 15 min and had positive inotropic action for 3 min after pulse injection. The results of *in vivo* and *in vitro* studies (rat and rabbit blood vessels, four types of dog vasculature) indicate, that vasodilatation of coronary arteries and vasodilatation of renal, mesenteric and femoral arteries at low concentrations (3 mg/ml) after administration of extracts of *Salviae Miltiorrhizae* might be ascribed to increased utilisation of extracellular calcium ions (activity of the drug plant on isolated blood vessels of rabbits was enhanced by 2 mM Ca^{2+}). According to Li *et al.* (1990), the vasodepressor effect, which might account for positive inotropic and for negative chronotropic effects (the latter through modulation of cholinergic activity) of *Salviae Miltiorrhizae* extracts, was probably angiotensin- and/or bradykinin-related.

When Chung *et al.* (1987) studied the effects of *Salviae miltiorrhizae* extract on renal function in normal rats, they established that this extract, after intraperitoneal single dose (10 mg/100 g body weight), markedly increased urine volume, and urinary urea, creatinine, sodium, potassium and inorganic phosphate excretion. No potassium retention was observed and no changes in the renin-angiotensin system or in aldosterone level were observed, what implies that natriuretic effect of extract was not mediated via reduced aldosterone secretion. Acute administration of *Salviae miltiorrhizae* extract also significantly increased glomerular filtration rate in renal plasma flow and increased renal blood flow, which might influence and increase in urinary urea and creatinine (Chung *et al.,* 1987). Aqueous extract of *Salviae miltiorrhizae radix,* when chronically administered to uremic rats with mild or moderate (but not severe) uremic state, was reported to decrease urea nitrogen, creatinine, methylguanidine and guanidinosuccinic acid levels and to increase serum guanidinoacetic acid concentration as well as to increase renal tissue blood flow while decreasing renal vascular resistance and blood pressure (Chung *et al.,* 1986; Yokozawa *et al.,* 1987). These observations indicate, that neurological and humoral factors responsible for lowering blood pressure may mediate an increase in renal blood flow. Diminished renal vascular resistance and attendant increase in renal blood flow by *Salviae miltiorrhizae radix* extract may contribute to the increase of glomerular filtration rate and the increase in urinary

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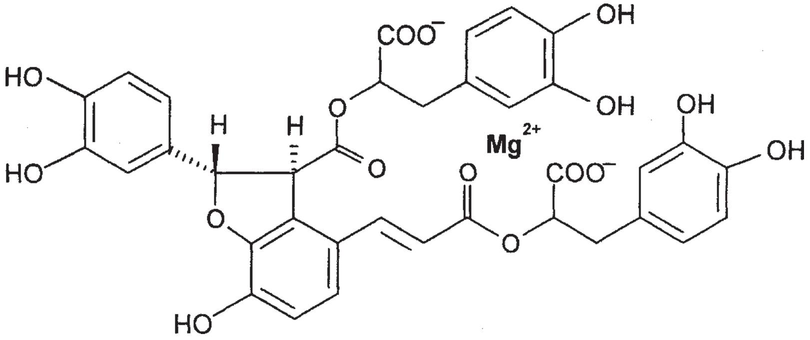

Structure 21

excretion of uremic toxins such as urea and creatinine. In the eighties, magnesium lithospermate B *(structure 21),* a tetramer of caffeic acid, was isolated from *Salviae miltiorrhizae radix* and was proved to be responsible for most of the above mentioned effects that facilitate renal function in normal rats or in rats with mild or moderate renal failure. Magnesium lithospermate B was found to cause significant decrease of urea nitrogen, creatinine, inorganic phosphate, methyl guanidine and guanidinosuccinic acid levels in blood of adenine diet-induced uremic rats and showed remarkable improving effect on uremie symptoms of these rats (Tanaka *et al.,* 1989; Yokozawa *et al.,* 1989b; Yokozawa *et al.,* 1990a).

After intraperitoneal administration of magnesium lithospermate B (10 mg/kg) to rats with adenine-induced renal failure, the levels of glomerular filtration rate, renal plasma flow and renal blood flow increased, while renal vascular resistance decreased (Yokozawa *et al.,* 1989a; Yokozawa *et al.,* 1989b; Yokozawa *et al.,* 1990a). Also, urinary excretions of prostaglandin E_2 (PGE_2) and 6-keto-prostaglandin F_1 (6-keto-PGF1), which have vasodilating effects on mesangial cells of glomeruli and on small vessel system of kidney, increased in magnesium lithospermate B treated rats, while there were no significant changes in excretion of vaso-constrictive prostanoid thromboxane B_2 (TXB_2). Significant increase in PGE_2 and 6-keto-PGF_1 were achieved also in normal rats, after intraperitoneal administration of magnesium lithospermate B (at 10 mg/kg body weight), while its effect became weaker as renal failure progressed due to the prolonged administration of adenine (24 days). Urinary sodium excretion decreased gradually with the progression of renal failure, whereas administration of magnesium lithospermate B significantly increased urinary sodium as well as potassium excretion together with a decrease in mean blood pressure, increase in renal tissue blood flow and increase in cerebral blood flow (Yokozawa *et al.,* 1992; Yokozawa *et al.,* 1993). Increase in renal tissue blood flow was associated with a significant increase in excretion of urinary urea, creatinine and inorganic phosphate. These results suggest that magnesium lithospermate B reduces renovascular hypertension by improving urinary electrolyte excretion and haemodynamics. These effects might be due to magnesium lithospermate B-influenced increase in formation of

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prostaglandin E_2 in renal tissue (which contribute to improvement of renal blood flow and to reduction in renal vascular resistance) and have a protective effect against renal failure providing renal tissues are still functioning.

Kidneys play an important role in the pathogenesis of hypertension as a consequence of a primary defect in renal haemodynamics that influences retention of fluid and electrolytes. It was found that magnesium lithospermate B does not affect renin-angiotensine-aldosterone system (Yokozawa *et al.,* 1990 b), but induces dilation of blood vessels, increase in renal blood flow and improvement of renal func tion by enhancing production and secretion of PGE_2 in kidney through activation of the kallikrein system (Yokozawa *et al.,* 1994). The kinin-kallikrein system, together with the prostaglandin system, is involved in the mechanism of blood pressure regulation and regional blood flow as well as the metabolism of water and electrolytes. Oral administration of magnesium lithospermate B (10 mg/kg) in spontaneously hypertensive rats resulted in significant decrease of systolic, mean and diastolic blood pressures, effects having depended on duration of administration period. Also, the low urinary kallikrein level in spontaneously hypertensive rats increased with a parallel increase in excretion of PGE_2, sodium and potassium. These findings suggest that magnesium lithospermate B, which proved to be a safety compound when administered orally (LD50 > 3000 mg/kg), reduces hypertension by improving renal circulatory state, at least partly through activation of the kinin-kallikrein-prostaglandin system in kidney.

Guoji *et al.* (1994) report that the polysaccharide fraction isolated from *Salviae miltiorrhizae radix,* which contains a large amount of uronic acids, reduces the symptoms of aminonucleoside (puromycin, PA)-induced experimental nephrosis in rats. A decreased urinary protein excretion, increased serum albumin and less severe lesions of the epithelial cells were observed in rats treated with PA (60 mg/kg, *i.v.*) after both oral (40 mg/kg) or intramuscular (2.5 mg/kg) injection of polysaccharide active fraction (AF). According to Guoji *et al.* (1994), this improvement was probably influenced by polyaninonic nature of AF due to many carboxyl groups of galacturonic acids, that might stabilise and restore glomerular basement mebrane in PA-induced nephrosis.

The hexane extract from *S. miltiorrhiza* root was shown to have strong antioxidant properties, similar to those of dihydrotanshinone I, isolated from non-polar extracts of Danshen (Gordon and Weng, 1992). Sodium tanshinone II-A sulfonate (STS) is a water-soluble derivative of tanshinone II A and displays marked cardiovascular activity. After STS-treatment of patients with cardiovascular diseases and cerebral thromoboembolism the symptoms like anginal pain and feeling of chest tightness were reduced and ischemic alterations in the electrocardiogram (EGG) showed a more normal course (Chen *et al.,* 1979; Xiao and Fu, 1987). Lithospermic acid B, when infused at 5.5 mol/kg into post ischemic rabbit heart, reduced the myocardial damage found in saline control by 62 10% (Fung *et al.,* 1993). STS was found to significantly reduce the myocardial infarct in a rabbit 1-hr ischemia and 3-hr reperfusion model (Wu *et al.,* 1993). STS did not inhibit oxygen uptake by xantine oxidase (XO) which is a key enzyme in free radical generation. Also, STS significantly prolonged the survival of cultured human saphe nousvein endothelial cells but not human ventricular

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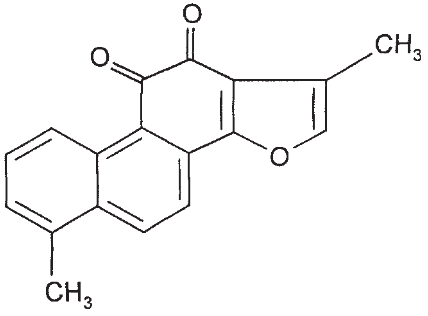
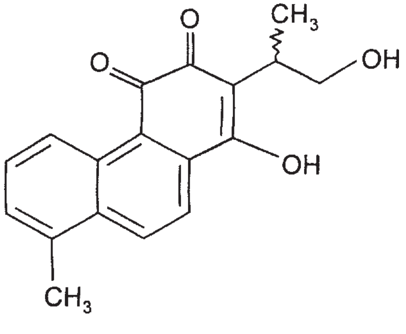
myocytes *in vitro* when these cells were separately exposed to XO-generated oxyradicals. STS was found to be a cardioprotective substance, which may beneficially influence vascular endothelium, a key site of oxidant generation and heart attack.

Bai and Wang (1994) clinically studied the haemodynamic effects of *Salvia miltiorrhiza* and compared it to nitroglycerin, and established that both drugs had similar vaso-dilating effects. Both drugs reduced the filling pressure of the left ventricle and increased the cardiac output, although the effect of *Salvia miltiorrhiza* was markedly superior and was more persistent than that of nitroglycerin.

Based on preclinical studies, the aqueous extract of *S. miltiorrhiza* was found to significantly reduce the mortality rate and to have a protective role in chemically (isoproterenol or $BaCl_2$) induced acute myocardial ischemia and arrhythmia (Cheng *et al.,* 1990), and in cardial ischemia induced by ligation of the coronary artery (Cheng *et al.,* 1992). Tanshinones are reported to protect myocardium against disturbances in cardiac function and metabolism induced by oxygen deficiency (Yagi *et al.,* 1989; Takeo *et al.,* 1990; Yagi *et al.,* 1991; Yagi *et al.,* 1994). When isolated rat hearts were subjected to hypoxic perfusion (20 min) in the presence of either 37.5 nM tanshinone I *(structure 22),* 29.5 nM cryptotanshinone *(structure 5),* or 37.5 nM tanshinone VI *(structure 23),* and tanshinone VI derivatives (42 nM), the decreased



Structure 22



Structure 23

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cardiac contractile force was significantly recovered after subsequent 45 min heart reoxygenation, while little recovery of cardiac contractile force was observed upon reoxygenation in control rats. Tanshinones prevented the hypoxia/reoxygenation-induced increase in tissue sodium and calcium and decrease in tissue potassium and magnesium. Also, resting tension (a marker for cardiac contractile failure after oxygen deficiency) at 45 min reoxygenation in hearts pre-treated with these compounds was significantly lower than that without treatment. Concomitantly, tanshinones dimin ished the release of creatine kinase (CK) and ATP metabolites such as adenosine, inosine and hypoxanthine from hypoxic/reoxygenated hearts (Yagi *et al.,* 1994). Release of CK from myocardium is considered to be an indicator of cardiac cell necro sis or of an increase in cell membrane permeability, while release of ATP metabolites in hypoxia/reoxygenation or ischemia/reperfusion hearts serves as an indicator of loss of purine nucleosides from myocardium. These results suggest that enhanced recovery of contractile force of rat heart upon reoxygenation by tanhinones may be at least partly due to restoration of heart tissue ionic concentrations, prevention of cardiac cell necro sis, preservation of cell membrane integrity and due to the improvement of restoration of myocardial high-energy phosphates in myocardium, which in turn may enhance restoration of ATP when oxygen is replenished.

Free radicals play an important role in pathogenesis of acute viral myocarditis (AVM). A clinical study of 60 children with AVM showed that *S. miltiorrhiza* as an effective antioxidant significantly decreased the levels of plasma lipid peroxide (LPO) and that of erythrocyte membrane microviscosity (EMMV) (Meng *et al.,* 1992). In most patients treated with *S. miltiorrhiza,* LDH, GOT and EGG recovered after one course. These results announce the myocardium- protective effects of the drug in acute viral myocarditis. Another clinical study on infantile acute toxic myocarditis also showed an efficiency of *Salvia miltiorrhiza* treatment and its advan tages over the western medicine control group, that can be seen in a significantly shorter period of hospitalisation and EGG normalisation when compared to the control group (Wang, 1993).

As stated above, cardiovascular activity is almost exclusively attributed to *Salvia miltiorrhiza* and its preparations. However, animal studies show that also *Salvia officinalis* posses the potential in lowering the blood pressure in animal studies (Newall *et al.,* 1996; Todorov *et al.,* 1984). When applied intravenously or duo denally, the aqueous-alcoholic extract of *S. officinalis* induced a moderate but prolonged lowering of blood pressure in cats.

BIO-ANTIOXIDATIVE, ANTIINFLAMMATORY AND TUMORIGENESIS-PREVENTING ACTIVITIES

Within the last decade, the importance of free radicals in the aetiology of disease has been increasingly recognised and has led to development of new approaches in biochemical evaluation of events associated with mutagenesis, tumorigenesis and/or cancer promotion. Biomembranes (microsomes, plasma membrane…) are rich in polyunsaturated fatty acids, which are very sensitive to the peroxidative damage

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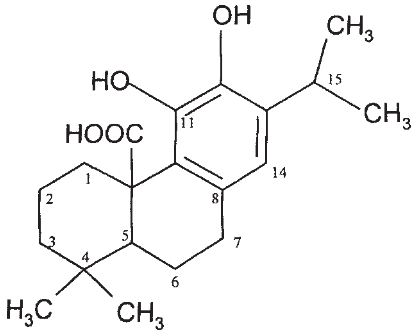
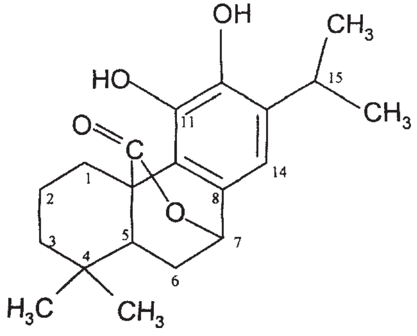
induced by free radicals. Free radicals are generated by metabolic pathways within the body or can also be caused by transformation of specific xenobiotic molecules and by ecological pollutants. Dietary supplies of natural antioxidants act as protective agents against such free radicals and can, in sufficient amounts, act as efficient scavengers of free radicals before any tissue damage occurs (Deighton *et al.*, 1993).

Leaves of sage (*S. officinalis* L.) are well known for their phenolic structure-based antioxidative potency (Chipault *et al.*, 1956; Farag *et al.*, 1989; Lamaison *et al.*, 1990; Schwarz and Ternes, 1992; Cuvelier *et al.*, 1994). Commercially available extracts of sage are mainly utilised by the food processing industry, but may be applicable in human health. Main sage phenolic diterpenes, which show high antioxidative activity are carnosic acid *(structure 24)*, which is known for its instability, and its degradation derivatives carnosol *(structure 25)*, rosmanol *(structure 26)*, its isomer epirosmanol *(structure 27)*, 7-methyl-epirosmanol (Cuvelier *et al.*, 1994; Schwarz *et al.*, 1992; Schwarz and Ternes, 1992) as well as rosmanol 9-ethyl ether (Markovic *et al.*, 1996). Rosmarinic acid *(structure 28)* also



Structure 24



Structure 25

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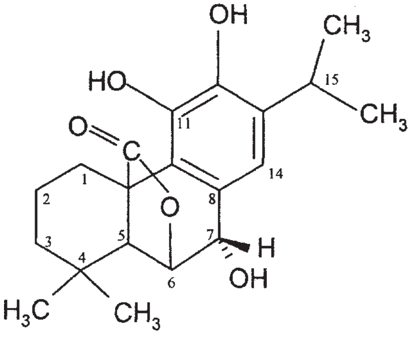
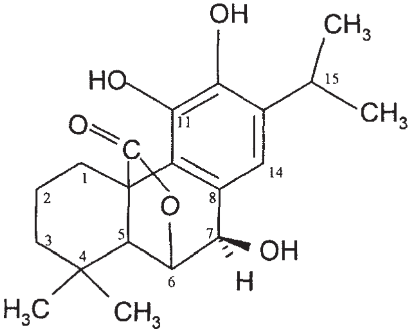
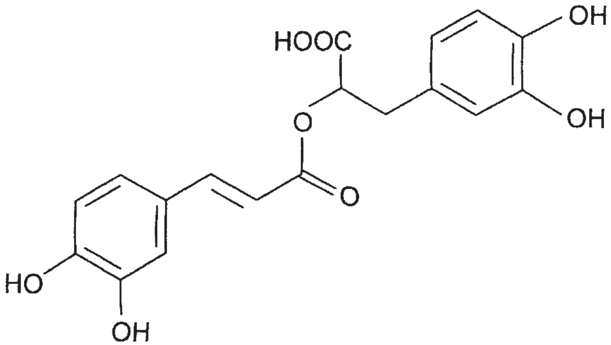


Structure 26



Structure 27



Structure 28

accounts for the antioxidant activity of sage. When measuring the radical scavenger effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical, the antioxidative effect of rosmarinic acid (EC_{50}=2.7 μg/ml) was comparable to that of ascorbic acid (Lamaison *et al.*, 1991). A variety of plant phenolic compounds were investigated for

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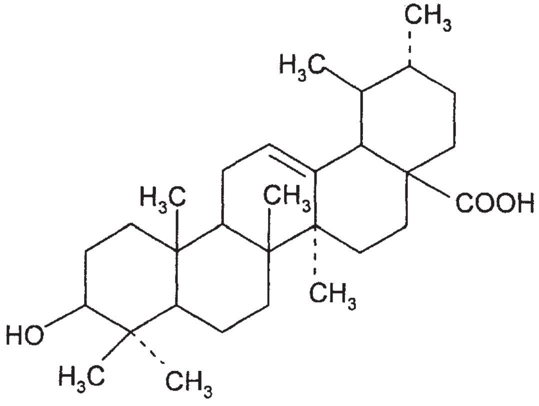
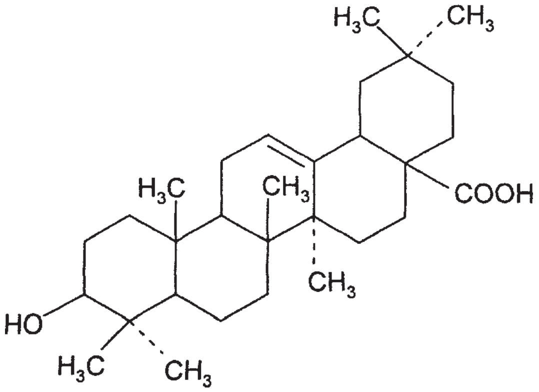


Structure 29

their antioxidative potential using human aortic endothelial cells (HAEC) to mediate oxidation of low-density lipoprotein (LDL) (Pearson *et al.,* 1997). All anti-oxidants produced a dose-dependent inhibition of LDL oxidation. Most potent antioxidants in HAEC system were carnosic acid, carnosol and rosmarinic acid.

Some strong natural antioxidants like carnosol were proved to exhibit anti-inflammatory and inhibitory effects with regard to tumor-initiation activities in mice test systems (Huang *et al.,* 1994). Also some sage compounds (ursolic and/or oleanolic acid) that show no antioxidant activity (Wu *et al.,* 1982) may turn promising in future research of inflammation and of cancer prevention. A squalene derived triterpenoid ursolic acid *(structure 29)* and its isomer oleanolic acid *(structure 30)* (up to 4% in sage leaves, dry weight basis) (Brieskorn and Kapadia, 1980), act anti-inflammatory and inhibit tumorigenesis in mouse skin (Tokuda *et al.,* 1986; Huang *et al.,* 1994; Ho *et al.,* 1994). Recent data on the anti-inflammatory activity of sage (*S. officinalis* L.) extracts when applied topically (ID_{50}= 2040 µg/cm^2) and evaluated as oedema inhibition after Croton oil—induced dermatitis in mouse ear, confirm/ suggest ursolic acid to be the main active ingredient, responsible for sage anti-inflammatory effect (Baricevic *et al.,*



Structure 30

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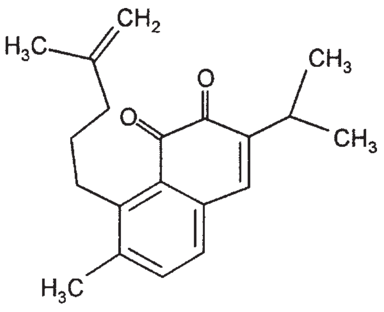

Structure 31

2000). The data on the pharmacological effects of these metabolites promise new therapeutic possibilities of sage extracts.

The importance and therapeutic potential of naturally occurring o-naphthoquinones, compounds that might be closely involved in inflammatory process inhibition, has been stressed. It has been reported (Hernández-Pérez *et al.,* 1995), that naphthoquinone derivatives of *S. aethiopis* have a similar pharmacological profile as NSAI (Non-Steroidal-Anti-Inflammatory) substances with regard to reducing oedema induced by carrageenan and contractions induced by phenyl-p-quinone. An o-naphthoquinone diterpenoid, aethiopinone *(structure 31)*, isolated from *S. aethiopis* L. roots, showed strong anti-inflammatory and antinociceptive effects in rodents' model systems and increased bleeding time in mice with similar potency as some of NSAI drugs (Hernández-Pérez *et al.,* 1995). In anti-inflammatory studies, measured by inhibition of Carrageenan paw oedema in mouse, aethiopinone at 100 mg/kg *p.o.* inhibited oedema formation similarly to NSAI drugs (Aspirin, Ibuprofen, Piroxicam) used as reference at 50 mg/kg *p.o.* Also in the TPA-induced ear inflammation model, aethiopinone significantly reduced ear oedema induced by phorbolic ester, when administered topically (but not orally) at 1.0 mg/ear. It is therefore as effective as some NSAI drugs, but less effective than steroidal reference drugs used at a dose as low as 0.1 mg/ear. Aethiopinone exerted its analgesic effect especially against thermal painful stimuli measured by tail immersion test (significant increase in reaction time of mice at 100 mg/kg p.o.), indicating the presence of a central analgesic action, although a moderate peripheral analgesia, was also conformed by the phenylquinone writhing test, when 100 mg/kg of aethiopinone was administered orally. Even though the structure of aethiopinone is similar to that of tanshinones, which had been reported as antipyretic agents, no such properties were found when aethiopinone was assayed against yeast-induced hyperthermia in rats.

Ursolic acid showed significant cytotoxicity in lymphatic leukemia cells P-388 (ED_{50}=3.15 µg/ml) and L-1210 (ED_{50}=4.00 µg/ml) as well as human lung carcinoma cell A-549 (ED_{50}=4.00 µg/ml) (Lee et *al.,* 1987; Fang and Mc Laughlin, 1989). Both carnosol and ursolic acid are referred to as being strong inhibitors of 12-O-tetradecanoylphorbol-13-acetate (TPA)-induced ornithine decarboxylase activity and of TPA-induced tumor promotion in mouse skin. The tumorigenesis-prevention potential of ursolic acid was comparable to that of retinoic acid (RA)—a known

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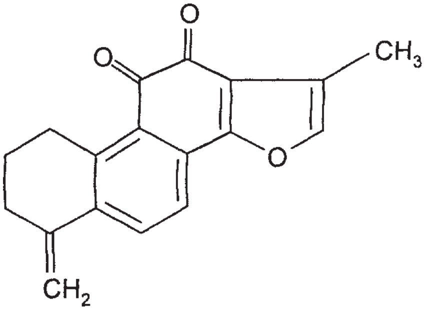
inhibitor of tumor promotion (Tokuda *et al.,* 1986; Huang *et al.,* 1994). Both ursolic acid- and oleanolic acid- treatment (41 nmol of each), when applied continuously before each TPA-treatment (4.1 nmol), delayed the formation of papillomas in mouse skin, significantly reduced the rate of papilloma-bearing mice and reduced the number of papillomas per mouse, when compared with the control group (only TPA treatment). Ursolic acid acted more effectively in a single application before initial TPA-treatment when compared to the effect of RA and/or oleanolic acid. So, the mechanism of the inhibitory action of ursolic acid (inhibition of the first critical cellular event in tumor promotion step caused by TPA) may differ slightly from those of RA and/or oleanolic acid, which block a critical second stage process in tumor promotion by TPA (induction of ornithine decarboxylase and polyamine levels).

A possible tumorigenesis preventing effect can be predicted for abietane diterpene galdosol (structure 1), isolated from *S. canariensis* L., which showed significant cytostatic activity (ID_{50}=0.50 µg/ml) when inhibition of development of single-layer culture of HeLA 229 cells was measured in *in vitro* experiment (Darias *et al.,* 1990).

One of the most dangerous environmental sources of cytogenetic damage is ionizing radiation, which acts either directly or by secondary reactions and induces ionization in tissues. Interaction of ionizing radiation with water and other protoplasmatic constituents in oxidative metabolism causes formation of harmful oxygen radicals. DNA lesions, caused by reactive oxygen species in mammalian cells are the initial event which may lead to possible mutagenesis and/or carcinogenesis and form the basis of spontaneous cancer incidence (Hanawalt, 1998; Lutz, 1998). Free radi cals play an important role in preventing deleterious alterations in cellular DNA and genotoxic effects caused by ionizing radiation in mammalian tissues. Many drugs and chemicals (for example sulfhydryl compounds) are known to increase the survival rate in animals. Based on animal models studies, *S. miltiorrhiza* and its extracts were shown to have a potential to prevent X-radiation-induced pulmonary injuries (Du *et al.,* 1990) and high dosage gamma-irradiation-induced platelet aggregation lesions (Wang *et al.,* 1991).

The antiproliferative activity of tanshinones against five human tumor cells, i.e. A-549 (lung), SK-OV-3 (ovary), SK-MEL-2 (melanoma), XF-498 (central nerve



Structure 32

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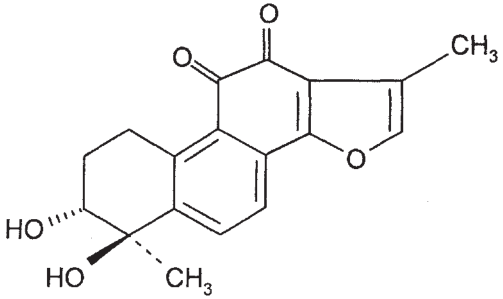


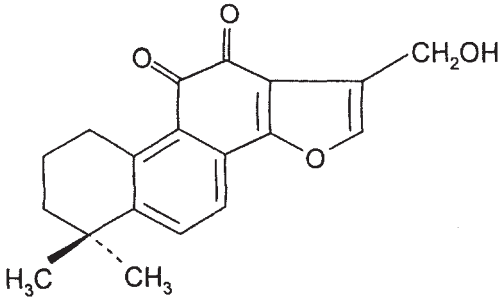
Structure 33

system) and HCT-15 (colon), was evaluated by sulfrhodamine-B method (Ryu *et al.*, 1997). 18 isolated tanshinones exhibited significant but presumably nonspecific cytotoxicity against all tested tumor cells, which might be attributed to common naphtoquinone skeleton rather than to substituents attached to it. Methylenetanshi quinone *(structure 32)* and tanshindiol C *(structure 33)* exhibited most powerful cytotoxic effects against tested tumor cells, with IC_{50} ranging from 0.4 µg/ml in A-549 cells to 2.2 µg/ml in SK-MEL-2 cells and IC_{50} from 0.3 µg/ml in SK-MEL-2 cells to 0.9 µg/ml in SK-OV-3 cancer cell lines respectively.

From *S. przewalskii* Maxim, var. *mandarinorum* Stib., a strong bacteriostatic compound, przewaquinone A *(structure 34)* was isolated. Przewaquinone A was reported (Xiao and Fu, 1987) to possess potential for inhibiting Lewis lung carcinoma and melanoma B-16.

ANTIMUTAGENIC ACTIVITY

Studies of bio-antimutagenesis, with emphasis on natural antimutagens from sage began in the late 80-ies when water extracts of *S. officinalis* were tested for their ability to suppress mutagenicity towards *Salmonella typhimurium* TA 98 of Trp-P-2,



Structure 34

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a carcinogen which occurs in some foods. Sage water extracts suppressed the mutagenicity of Trp-P-2 by 90% (Natake *et al.,* 1989). To assess the antimutagenic potential of sage (*S. officinalis* L.) extracts, our laboratory used a *E. coli* test system involving the repair proficient strain WP2 *(trp)* and the excision repair deficient strain WP2uvrA. Methanolic extracts applied at nontoxic doses reduced the number of UV induced revenants in both strains, but the reduction was significantly more efficient in the repair proficient strain. The extracts were further tested for the abillity to reduce spontaneous mutation rate in mismatch repair defficient mutator strains *E. coli* IB101, *E. coli* IB 102 and *E. coli* IB 103 and non of the tested extracts reduced spontaneous mutation rate. (Baricevic *et al.,* 1996; Filipic and Baricevic, 1997).

These results are in agreement with previous data on suppression of UV-induced mutation frequency in E. *coli* repair proficient strains with ethanolic extracts of cultivated sage, that were not active in repair deficient strains (Vukovic-Gacic *et al.,* 1993). The potential antimutagenic effect of extracts of cultivated and wild *Salvia officinalis* was investigated also on new *E. coli* K12 reversion assay system for identifying antimutagens. Among three extracts tested, only extract 1, with the highest content of monoterpenoid camphor, showed bio-antimutagenic effect. Extract 1 or camphor alone suppressed UV induced mutagenesis when tested with the *E. coli* repair proficient strain, while no effect was observed when tested with the mismatch repair deficient strains (Simic *et al.,* 1997). The results obtained by comparing model bio-antimutagens with sage extracts on the same *E. coli* K12 assay system indicate, that bio-antimutagenic agents from cultivated sage enhance error-free recombina tional DNA repair by intervening in a formation of RecA-DNA complex and channelling it into recombination reaction (Simic *et al.,* 1997; Simic *et al.,* 1998). Results of the study, where several plant extracts were tested for their effect on UV-induced beta-galactosidase activity (SOS gene expression) proved no antimutagenic potential of sage extract. Moreover, the level of UV-induced enzyme was even higher after addition of sage extract (Vukovic-Gacic *et al.,* 1993; Simic *et al.,* 1997).

Recently we showed that n-hexane and chloroform sage extracts inhibited UV induced SOS response in *Salmonella typhimurium* TA1535/pSK1002 (Filipic and Baricevic, 1998). Contrary to our results Simic *et al.* (1997) observed no effect of ethanol sage extracts on UV induced SOS response. Possible reason for these differencies might be, that different active principles were isolated due to the different extraction procedures used by the two groups. Another reason might be due to the fact that different test systems having different target genes for detection of SOS response were used. In our laboratory we used *Salmonella typhimurium* TA1535/pSK1002 that has the *umuC-lacZ* fused gene while Simic *et al.* (1997) used *E. coli* IB100 that has the *sfiA-lacZ* fused gene.

Based on these results, bio-antimutagens from sage extracts could be used as preventive agents in intervention strategies against cancer but further investigation on active principles of the extracts is needed.

Mutagenic and/or antimutagenic effects are reported also in *S. miltiorrhiza* Bge. 4 tanshinones (dihydrotanshinone I, cryptotanshinone, tanshinone I, and tanshinone IIA), isolated from ether extract of *S. miltiorrhiza,* were recognised to be modulators

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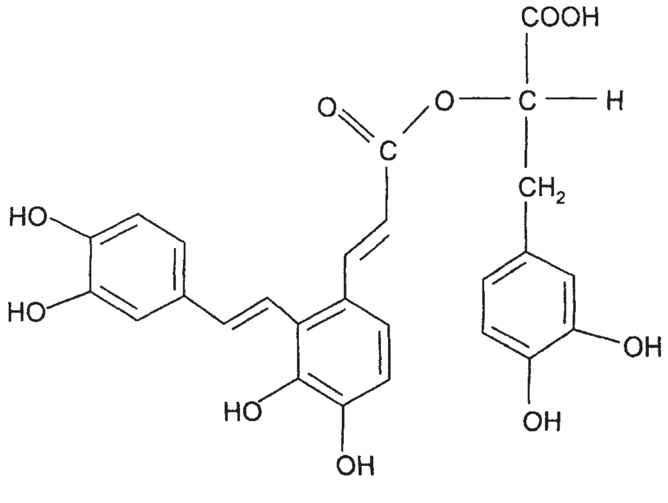
of Trp-P-1 and BP (benzopyrene) mutagenic activities in *Salmonella typbimurium* TA98. They enhanced both mutagens at low concentrations by 8 to 24-fold at 20 g/ plate), but suppressed mutagens at high concentrations. Dihydrotanshinone I suppressed Trp-P-1 activity completely at 100 µg/plate (Sato *et al.*, 1992).

PEPTIC-ANTIULCER ACTIVITY

Salvianolic acid A (Sal A) *(structure 35)* was found to be a strong inhibitor of gastric H^+, K^+-ATPase and to be effective in inhibition of acid secretion and in inhibition of stress-induced gastric lesions (Murakami *et al.*, 1990). Hydroxyl groups were identified as an important moiety of Sal A in competitive interaction with ATP, thereby reducing the phosphorylation of gastric enzyme, responsible for acid secretion. Sal A was about 10 times stronger as H^+, K^+-ATPase inhibitor but less effective in antisecretory and antiulcer activities than the well known anti-ulcer agent omeprazole. This was probably due to metabolic changes at hydroxyl groups.

ANTISPASMODIC ACTIVITY

Also, antispasmodic action *in vitro* has been reported for sage (*S. officinalis* L. and *S. triloba* L.) extracts, which inhibited smooth-muscle contractions induced by acetylcholine, histamine, serotonin and barium chloride by 60 to 80%. Contrary to this, a same experiment with vervain sage (*S. verbenacea* L.) extracts showed, that this species increased spasmogenic effect of applied spasmogens on isolated smooth muscle segments of guinea-pig ileum (Todorov *et al.*, 1984). Although some of sage



Structure 35

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essential oil components like pinene (Taddei *et al.,* 1988) or borneol (in higher doses) (Cabo *et al.,* 1986) show spasmogenic activity *per se,* dose-dependent anti-spasmodic activities of sage essential oil *in vitro* (guinea pig ileum) (Taddei *et al.,* 1988) and *in vivo* (Giachetti *et al.,* 1986) have been reported. Camphor and borneol from the essential oil of *S. lavandulifolia* Vahl., were tested for spasmolytic activity on isolated rat duodenal tissue and showed significant inhibitory activity against at least one of the chemical spasmogenic agents ($BaCl_2$ and acetylcholine) (Cabo *et al.,* 1986).

According to Giachetti *et al.* (1988) intravenous injection of *S. officinalis* essential oil emulsions resulted in a partial (10–25 mg/kg) or total (50 mg/kg) unblockage of contracted guinea pig Oddi's sphincter, induced by intravenous morphine hydrochloride (1 mg/kg *i.v.*).

HYPOGLYCAEMIC ACTIVITY

Based on ethnopharmacological data and pharmacological studies, *S. officinalis* L. (Essway *et al.,* 1995), *S. lavandulifolia* Vahl. (Jimenez *et al.,* 1985; Zarzuelo *et al.,* 1990), *S. triloba* L.(Yaniv *et al.,* 1987; Perfumi *et al,* 1991) and *S. aegyptyaca* (Shabana *et al.,* 1990) possess strong hypoglycaemic properties. In *S. officinalis* essential oil (1950 mg/kg, *i.p.*) was tested and proved to be hypoglicaemically active in normal or in alloxan-induced diabetic rats. Results with laboratory rats treated with *S. lavandulifolia* Vahl. aqueous extract indicate, that hypoglycaemic action may be a result of several synchronous mechanisms (Zarzuelo *et al.,* 1990). These include potentiation of insulin release induced by glucose, increased peripheral uptake of glucose, decreased intestinal absorption of glucose. In case of chronic treatment, hyperplasia of pancreatic islet beta cells was suggested to act as physiological background for the hypoglycaemic activity of the *S. lavandulifolia* aqueous extract.

Water extracts of leaves of *S. triloba,* used in folk medicine of the eastern Mediterranean regions as a hypoglycaemic agent, were assayed on normoglycaemic rabbits and in rabbits made hyperglycaemic by alloxan administration (Perfumi *et al.,* 1991). Oral dose of 0.250 g/kg body weight caused a statistically significant reduction in blood glucose levels in alloxan-hyperglycaemic rabbits, but not in normoglycaemic animals. Contrary to this, hypoglycaemic effect was induced by single oral dose of water extract in both normoglycaemic and alloxanhyper glycaemic rabbits orally loaded with glucose. However, in these animals the *S. triloba* extract did not modify plasma insulin levels. The hypoglycaemic effect of the drug was not demonstrated in rabbits which received glucose load intravenously. These data suggest that the *S. triloba* treatment produces hypoglycaemia mainly by reducing the intestinal absorption of glucose.

HEPATOPROTECTIVE EFFECTS

The damage of cell biomembranes integrity (due to lipid peroxidation of their unsaturated fatty acids) caused by free radicals is considered as a pathway in some experimental liver injuries and clinical liver diseases. Various factors have been reported to injure liver, and especially free radicals derived from oxygen and other

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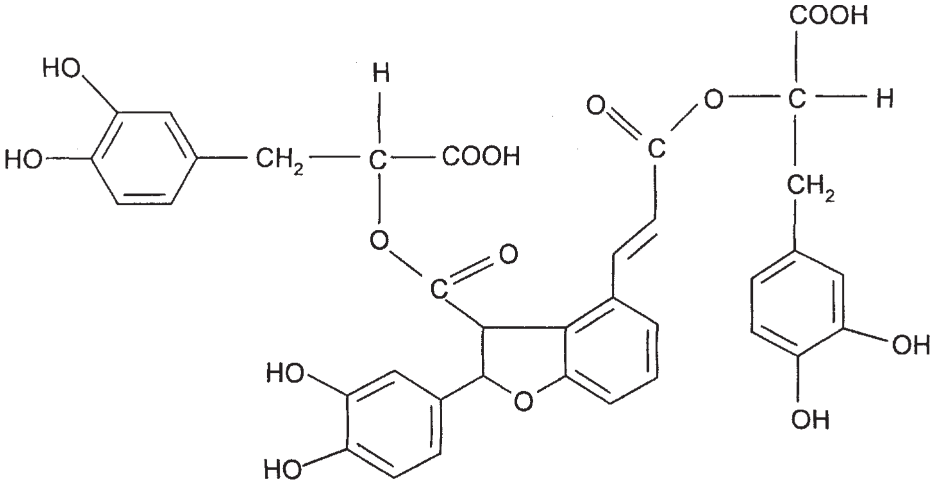
chemicals are thought to be strong noxious agents. Therefore, it can be assumed that drugs with antioxidative properties might be effective in protecting the liver against oxidative stress-induced injuries. Many data on the beneficial effects of natural compounds in experimental liver injuries support this hypothesis.

For example, a strong radical scavenging effect on 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical and inhibitory activity against free radical generation as well as cytoprotective effect against t-BHP in cultured liver cell serve as examples of anti oxidative potential of methanolic extract of *S. miltiorrhiza* Bge. roots (Kang *et al.*, 1997). Also aqueous extract of *S. miltiorrhiza* roots was reported to have scavenging oxygen free radical activity and to act protectively against liver injury caused by chemicals such as carbon tetrachloride (CCl_4) (Yang *et al.*, 1990; Yu *et al.*, 1992). Liu *et al.* (1992) studied effects of seven phenolic compounds isolated from aqueous extract of *S. miltiorrhiza* on peroxidative damage to liver microsomes, hepatocytes and erythrocytes of rats. Among tested phenolic compounds, the action of salvianolic acid (Sal A) against peroxidative damage/MDA production of rat liver microsomes and hepatocytes (induced by iron/cysteine and Vitamin C/NADPH) and against hemolysis of rat erythrocytes (induced by H_2O_2) was the most potent. The site of protective action of Sal A against peroxidative damage is thought to be at the initiation stage of lipid peroxidation of polyunsaturated fatty acids of bio membranes. The potency of Sal A biomembranes-protecting activity can be explained by multiple phenolic hydroxyl groups. Also, the antioxidant activity of some other compounds like Vitamin E and butylated hydroxyl toluene is closely related to the existence of a phenolic hydroxyl group. Another water soluble polyphenolic antioxidant, salvianolic acid B (Sal B) *(structure 36)*, which was also isolated from the roots of *S. miltiorrhiza*, was likewise found to scavenge DPPH. Sal



Structure 36

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B prevented endothelial damage by its antioxidant potential, increased the content of vitamin E in LDL, inhibited LDL oxidative modification in hypercholesterolemic animals, and had plasma cholesterol-lowering effect (Wu *et al.,* 1998).

Further studies show lithospermate B to be an active constituent of the water extract of *S. miltiorrhiza* which inhibits experimental liver injuries, induced either chemically (CCl_4^-) or immunologically (D-galactosamine/lipopolysaccharide, D-GalN/LPS, an active principle of endotoxin) (Hase *et al.,* 1997). Lithospermate B, which contains two carboxylic groups and seven phenolic hydroxy groups, was identified as a mixture of magnesium and calcium (3:1) salts of lithospermic acid B. Lithospermate B (0.1–100 µg/ml) showed a concentration-dependent protective effect on CCl_4^-induced cultured hepatocytes necrosis (measured as aspartate aminotransferase (AST) concentrations in *in vitro* medium 60 min after CCl_4^- challenge). The effect of lithospermate B (at 5–10 µg/ml) was more potent than that of glycyrrhizin (at 10 µg/ml), a clinically used drug for liver diseases in Japan. Results of *in vivo* experiments indicate, that lithospermate B significantly protect both CCl_4-induced liver injury in rats (at 50 and 200 mg/kg), measured as serum alanin aminotransferase (ALT), AST and lactic dehydrogenase (LDH) enzyme levels and D-GalN/LPS induced liver injury in mice (at 500 mg/kg *p.o.* or 50 mg/kg *s.c.*), measured as blood AST levels. The effects of lithospermate B in liver damage control in both animal studies were as strong as that of glycyrrhizin.

Both oleanolic acid and ursolic acid have antihyperlipidemic properties and were shown to be effective in protecting against chemically induced liver injury in laboratory animals (Liu, 1995). Multiple and toxicant-dependent mechanisms are believed to be involved in hepatoprotective effects of oleanolic acid, which protects many (e.g. CCl_4, acetaminophen, cadmium, bromo-benzene-furosemide, colchicine, D-galactosamine…) but not all of the hepatotoxicants. In comparison, ursolic acid is even more potent than oleanolic acid in decreasing the chemically induced liver injury. Suppression of hepatic cytochrome P-450 enzymes (inhibition of toxicant activation), enhancement of body defence systems, preventing liver lesions from progressing to fibrosis and stimulating liver regeneration are some of the important mechanisms of hepatoprotection.

The crude extract of *S. miltiorrhiza* dried roots and one of its main abietanoid diterpenes, tanshinone IIA, enhanced adenylate cyclase (AC) activity in purified rat liver plasma membranes in a progressive, time-dependent manner of stimulatory response (Bombardelli *et al.,* 1992).

Experimental evidence, i.e. demonstration of liver protection in test animals, has been presented for aerial parts of *S. plebeia,* a species traditionally used in folk medicine for treatment of hepatitis in Taiwan (Lin and Kan, 1990).

CENTRAL NERVOUS SYSTEM ACTIVITY

In cat model experiments, the extract of *S. miltiorrhiza* Bge. inhibited discharges of visceral pain in posterior nucleus of thalamus. It was suggested that this analgesic effect was exerted through the central nervous system (Liu *et al.,* 1990). The Central Nervous System (CNS) inhibitory effects of Danshen extracts could be attributed to

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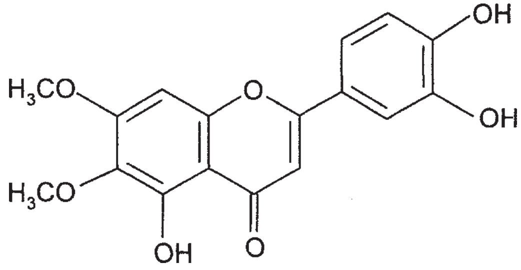

Structure 37

the interaction of active compounds with benzodiazepine (BDZ) sites of GABA receptors (Liao *et al.,* 1995). Several groups of researchers have reported the presence of benzodiazepine receptor ligands in extracts of plants, used in traditional medicine as anticonvulsants and tranquilizers (Nielsen *et al.,* 1988; Medina *et al.,* 1990; Wolfmann *et al.,* 1994; Viola *et al.,* 1994; Viola *et al.,* 1995). Active principles were found to be flavonoid derivatives with low micromolar affinities for brain BDZ receptor and in some cases, they were characterised as partial agonists, exhibiting selective anxiolytic, but not sedative properties (Wofman *et al.,* 1994; Viola *et al.,* 1995). Contrary to this, cirsiol (*structure 37*), a flavonoid isolated from aerial parts of *S. guaranitica* St. Hil., possesses cut-sedative and hypnotic properties, but shows no myorelaxant or anticonvulsant activities (Viola *et al.,* 1997). Cirsiol was shown to have a different pharmacological profile than some other naturally-occurring flavonoids (chrysin or apigenin), that specifically recognise central BDZ receptor, due to a differential interaction with a subpopulation of BDZ binding sites. Cirsiol was found to be a potent low affinity competitive ligand for type I benzodiazepine receptor in rat cerebral cortex. This interaction expresses sedative and hypnotic effects, but does not induce anxiolysis and muscle relaxation.

In some *in vitro* studies, diterpenes like carnosic acid and carnosol, which bind to the chloride channel of the GABA/benzodiazepine receptor complex in brain tissue, were considered as active inhibitory agents in the central nervous system (Rutherford *et al.,* 1992).

As shown in step-down and step-through tests, Sal A could at 3 and 10 mg/kg *(i.v.)* improve the impaired memory function induced by cerebral ischemia-reperfusion in mice (Du and Zhang, 1997). In the Sal A-treated group, a lower number of errors was observed and latency was longer than that of the control group. When administered intravenously at the same dosages Sal A was also found to reduce malondialdehyde contents in cortex, hippocampus and corpus striatum of cerebral ischemia-reperfusion rats *in vivo*. Sal A (10–100 nM) was shown to inhibit lipid-peroxidation of brain and to scavenge free hydroxyl radicals. These results suggest, that the protective effect in brain injury and ameliorating effect on learning and memory may be related to anti-oxidant activity of Sal A.Rosmarinic acid, lithospermic acid and its methyl ester derivatives are polyphenolic acids isolated from EtOAc fraction of methanolic extract of *S. miltiorrhiza,* and were proved to

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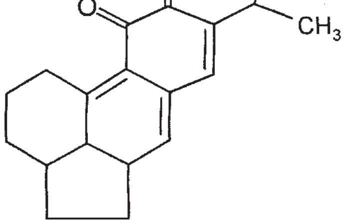
have strong inhibitory effects on adenylate cyclase (AC) in both rat brain and in rat erythrocytes (Kohda *et al.*, 1989). Lee *et al.* (1991) observed that abietane-derived diterpene quinones (tanshinones) from *S. miltiorrhiza* strongly inhibited binding of ^{3}Hflunitrazepam, radiolabeled benzo diazepine, to the central BDZ receptor in bovine cerebral cortical membranes. Benzodiazepines are synthetic psychotropic drugs, clinically used as sedatives/ hypnotics, muscle relaxants, anxiolytic and anticonvulsant drugs in the treatment of sleep disturbances, muscle spasms, anxiety, and convulsive disorders (Rall, 1990).

Among tanshinones isolated, miltirone displayed the highest potency in central BDZ receptor binding assay (IC_{50}=0.3 µM). It behaved as a partial agonist in central BDZ receptor binding and behavioural tests. Miltirone *(structure 17)*, when administered orally (10–60 mg/kg) to mice, showed tranquilizing activity. In contrast to diazepam (a full BDZ receptor agonist) miltirone produced no muscle relaxant effect. Chronic treatment of mice with miltirone (10 mg/kg, *p.o.*) twice daily in the period of 17 days did not cause sedation and did not induce drug dependence and withdrawal reactions. On the basis of these results, Chang *et al.* (1991) synthesised 22 related compounds in order to identify the key structural elements involved in interaction of miltirone with the central benzodiazepine receptor. It was found that quinone structure plays an important role in receptor interaction. Ring A and iso propyl group on ring C which can be replaced with a methyl group with minimal reduction in affinity, are essential moieties of miltirone for its interaction with the central BDZ receptor. When rings A and B were synthetically linked with an ethylene bridge, the analogue 89 *(structure 38)* was obtained, which showed higher potency in inhibiting of binding of ^{3}Hflunitrazepam to the central benzodiazepine receptor (IC_{50}=0.05 µM). Miltirone and its synthetic analogue 89 can be thus considered as potential non-sedative and non-addictive anxiolytic drugs.

PEST-TOXIC AND REPELLENT ACTIVITY

Aromatic plants and their essential oils are considered as the most effective new group of ecological products in insect and spider mite pest control. Many experiments have been carried out which show insecticidal/acaricidal and/or



Structure 38

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repellent potential of the *Salvia* genus plants. Essential oils and their monoterpenoids are the most prevalent active constituents. These show either fumigant (*S. triloba* L.) or topical toxicity (*S. cardiophylla* Benth., *S. triloba* L.) as well as antifeedant or repellent (*S. officinalis* L., *S. sclarea* L., *S. triloba* L.) effects when concentration is high enough (Polyakov *et al.,* 1977; Mansour *et al.,* 1986; Hirschfeld and Klingauf, 1988; Shaaya *et al.,* 1991; Konstantopoulou *et al.,* 1992; Schmeda-Hirschmann and Rojas de Arias, 1992; Lee *et al.,* 1997).

TOXICITY

Sage and its commercial preparations, when either inhaled or ingested, were found to provoke convulsions that originate in the central nervous system. This effect has been known for more than a century (Cadeac and Meunier 1881; Grimaud-Gaspari, 1979). Several cases of human poisoning accompanied by tonico-clonic convulsions as the major symptom were observed (Millet *et al.,* 1981). Based on the experimental study of sage neurotoxicity in rats, the subconvulsive limit dose of sage essential oil was 0.3 g/kg. Convulsions started at 0.50 g/kg and became lethal with 1.25 g/kg (Millet *et al.,* 1979). Daily repeated injection of subclinical doses of sage oil had cumulative toxic effects that resulted in electrocortical clonic seizures (Millet *et al.,* 1981). Furthermore, sage essential oil has a potency to reduce epileptogenic threshold and to facilitate kindling what was showed in rats experimental model of epilepsy (Dury *et al.,* 1986). The toxicity of sage oil is apparently caused by ketone terpenoids—thujone and camphor content, so the oil should not be ingested (Millet *et al.,* 1979, Millet *et al.,* 1981, De Vincenzi and Mancini, 1997).

When *Salvia* genus essential oil content was observed only a few species, i.e. *S. officinalis* L., *S. lavandulifolia* Vahl., *S. triloba* L. and *S. sclarea* L. had permanently high levels of it. Other species contained essential oil only in traces. Also, its content might have been beneath a detectable limit (Mate *et al.,* 1993; De Vincenzi and Maialetti, 1992; Hoppe, 1975). Although there exists a great variability in the composition of major constituents of essential oils, which depends on the origin of plants, it can be generally assumed that thujone and camphor are the prevalent components in *S. officinalis* essential oil. In *S. lavandulifolia* 1, 8-cineole, camphor, ß-pinene and sabinyl acetate prevail. In *S. triloba* 1, 8-cineole is the most prevalent with minor camphor and thujone content and in *S. sclarea* essential oil linalool, linalyl acetate, terpineol, geranyl acetate and sclareol were identified as major constituents (Hoppe, 1975; Lawrence, 1983; Putievsky and Ravis, 1985; Tucker and Maciarello, 1990; Sivropoulou *et al.,* 1997; Souleles and Argyriadou, 1997; Lawrence, 1998). Both thujone and camphor are known to be highly toxic if they are used in prolonged treatment. Especially camphor, even when ingested in small amounts or when its administration is associated with other factors (for example febrile seizures) can cause serious or fatal consequences in small children (Calvelli *et al.,* 1987; Galland *et al.,* 1992; Liebelt and Shannon, 1993; Theis and Koren, 1995). Given the toxicity of sage essential oil, also the crude drug or its extracts should be used carefully. Its interactions with other drugs can also be dangerous. Sage may interfere with existing hypoglycaemic and anticovulsant therapies and may

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potentiate sedative effects of other drugs (Newall *et al.,* 1996). Yu *et al.* (1997) report on the interaction of the extract of *S. miltiorrhiza* with warfarin, an anticoagulant drug used in the prevention of thromboembolic diseases. This interaction can provoke danshen-induced overcoagulation with severe abnormalities of clothing in patients with rheumatic heart disease.

Due to a high proportion of *a*- and *ß*- thujones in essential oil, which are known to possess abortifacient property, sage is contraindicated in pregnancy. Also, haemorrhoids and acute inflammation processes represent contraindication for use of sage or its preparations (Anonymus, 1994). Acute LD_{50} values for sage oil are documented as 2.6 g/kg (orally, rat) and 5g/kg (intradermal, rabbit) (Newall *et al.,* 1996). Because of its moderate skin irritating effects, sage oil is not recommended in aromatherapy (Newall *et al.,* 1996).

S. lavandulifolia Vahl. was also reported to be abortifaciently active, what is due to the relatively high content in sabinyl acetate in some of the chemotypes (Pages *et al.,* 1992; Fournier *et al.,* 1993). Recently an abortifacient property of *S. triloba* was observed, although compounds responsible for this effect have not as yet been discovered. Ingestion of S. *triloba* aqueous (800 mg/kg) or ethanolic extracts (400 mg/kg) in a duration of a longer period (30 consecutive days) reduced the number of implantations or viable foetuses and increased the number of resorbtions in pregnant rats (Elbetieha *et al.,* 1998). Crude drugs and their preparations should therefore be used with care during pregnancy.

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12. ANTIOXIDANTS FROM *SALVIA OFFICINALIS*

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Salvia officinalis (sage) is a perennial shrub native to southern Europe and Asia Minor (Embong *et al.,* 1977; Simon *et al.,* 1984). The plant is both cultivated and collected from the wild in Turkey, Italy, Greece, former Yugoslavia, Crete, Spain and France (Heath, 1974). There are about 700 species of *Salvia,* but only a few types are commercially important (Lawrence, 1981). *S. officinalis* L. (Dalmatian) serves as the standard sage to which others are compared as it is considered to possess the finest and most characteristic sage aroma (Small, 1997). The name *salvia,* coming from the Latin *salvare* "to heal or save", indicates the virtues attributed to this herb of restoring health and saving from sickness. It was extolled in a proverb: "He that would live for aye must eat sage in May". An infusion of *Salvia officinalis purpurescens* (red sage) can be used as an antiseptic gargle for sore throats. *Salvia scalrea's* common name "clear eye" may come from the word clary, or more likely from its use to remove obstructions from the eyes. The mucilage from the soaked seeds helps to do this, and to soothe the inflamed eyes (Hooper, 1984). Over the years, extensive research has been carried out worldwide in order to investigate the composition of sage volatile oil.

In 1991, Bernath and co-workers analysed essential oils from plants of *Salvia officinalis* and *S. sclarea* collected from cultivated areas in Hungary and for plants of *S. austriaca* and *S. aethiopis* collected from wild stands. The plant growth, development and DM production differed according to the environmental conditions and the particular species. The quantity of essential oil was influenced more by the environment than the species; the converse was true for the qualitative characteristics. Maximum DM and essential oil production occurred in the field and minimum levels under the cold, low-light regime; this difference was most marked for *S. officinalis* and *S. austriaca*. Under cold climatic conditions, there was a marked reduction in the contents of *a*-pinene and 1, 8-cineole compared with the composition of plants grown in the field or in warmth, but the contents of *ß*-pinene, *ß*-selinene and ledol remained stable in the different environments.

Differences in quantity and quality were also noted when Svoboda and Deans (1992) studied the variability of sage and rosemary volatile oils available on the British market. Samples of *Salvia officinalis,* both the dried herb and essential oil, were obtained from various dealers and suppliers of the British market. Both species were also cultivated in Scotland at Auchincruive, for comparison of the oil characteristics. Dried samples were hydrodistilled, and all samples were analysed

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using GC (gas chromatography). Herbal material was also examined under the lightmicro scope for purity and cleanliness. There were considerable variations between the samples. Scottish-grown material was of very good quality compared with the imported samples. The temperature of drying of plant material prior to distillation is critical to the quality of the subsequent oil. Recommendations were made for temperatures < 40 °C for retention of the more volatile components in sage (Deans and Svoboda, 1992), while geographic location has been shown to influence the chemical composition of sage (Putievsky *et al.,* 1992; Holla and Vaverkova, 1993).

The essential oil, steam-distilled from aerial parts of *S. officinalis* var. *angustifolia,* was analysed by GC-mass spectrometry (GC-MS) (Pace and Piccaglia, 1995). Thirty-four components were identified, monoterpenes being the most abundant (about 70%). The oil, characterized by a high content of *α*-thujone (39%) and considerable amounts of *ß*-pinene, 1, 8-cineole (eucalyptol), *ß*-caryophyllene and *α*-humulene.

The leaf essential oils of two wild species, *S. tomentosa* and *S. scabiosifolia,* collected at the flowering stage in Bulgaria, were compared with that of *S. officinalis* (Tsankova *et al.,* 1994). The essential oil of *S. tomentosa* was characterized by borneol (19.4%) and *ß*-pinene (29.1%). The essential oil of *S. scabiosifolia* was characterized by a high camphor content (48.7%). The main oil constituents of *S. officinalis* were *ß*- and *ß*-thujone (29.4 and 17.4%, respectively), which were not detected in the other essential oils, 1, 8-cineole (12.5%), and camphor (11.7%).

The essential oils of commercially available samples of leaves of *S. officinalis* (Photograph 1, 2) and *S. fruticosa* (used as medicinal and culinary herbs) obtained by steam distillation and dichloromethane extraction were analysed by GC (Langer *et al.,* 1996). Although standardized conditions of sample preparation were employed, differences in the composition of the oils were found: steam distillation yielded a reduced amount of the less volatile compounds, and the accuracy of determination was significantly lower than in the case of extraction. The commercial samples, which differed considerably in the composition of their essential oils, were quite heterogeneous in respect to their oil content partly due to the different ages of the leaves. Extraction of individual leaves of sage showed a decrease in the *a*-thujone content, with a corresponding increase in the relative amount of camphor, related to leaf age. Owing to the observed variability of the essential oil composition of sage, the relative contents of *a*-thujone, *β*-thujone and camphor have to be totalled in order to form a significant parameter for the characterization of *Salvia* species. This parameter varied between 45 and 68% in *S. officinalis* and between 4.8 and 15.9% in *S. fruticosa* with a small standard deviation. Consideration of this parameter, together with the amount of 1, 8-cineole (*S. officinalis* 2.8–23%; *S. fruticosa* 55–75%), permits the differentiation between these species and respective mixtures.

In recent years, however, problems and anxieties have arisen with the synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) whereby new long term studies have shown these moieties could produce tumours in animals whose diet was supplemented with these compounds (Kappus, 1991). The actual effect of BHA and BHT on human cancer risk is unknown. The amounts of BHA and BHT in the daily diet are considered to be safe (Kochhar, 1991), but the

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possibility of several synthetic antioxidants acting synergistically at high enough concentrations to cause cellular damage cannot be discounted. These concerns have lead to the interest in preparing antioxidants from natural sources with minimal processing. The presence of antioxidants in a number of different herbs and spices, specifically rosemary *(Rosmarinus officinalis)* and sage *(Salvia officinalis),* is well known. The discovery of antioxidants to increase the storage of foods has made possible the marketing of many new products and has directly benefited the consumers. Today, antioxidants are widely used in the food and pharmaceutical industries (Madsen and Bertelsen, 1995).

Cuvelier *et al.* (1994a) separated the major antioxidants in sage by high performance liquid chromatography (HPLC). Rosmarinic acid and carnosic acid, the major antioxidants present in sage, were identified and quantitatively determined on a single HPLC chromatogram, using an adapted gradient elution pattern. In the same year, the authors separated the antioxidant compounds of sage oleoresin by column chromatography and HPLC (Cuvelier *et al.,* 1994b). Six major compounds were purified and identified by IR, MS, and 1H NMR spectrometry as carnosol, carnosic acid, rosmadial, rosmanol, epirosmanol, and methyl carnosate (all of which, except rosmadial, are diterpenes of the ferruginol type with two orthophenolic functions and one isopropyl group on the adjacent carbon). Their antioxidative activity was measured with an accelerated test (based on the disappearance of methyl linoleate in a lipophilic solvent under strong oxidizing conditions), and their content was quantified in sage and in four commercial rosemary extracts. Carnosic acid showed the greatest antioxidant activity (Figure 1). High speed counter-current chromatography has also been successfully used in the separation and purification of sage extracts (Fischer *et al.,* 1991) while elevated levels of carnosic acid have been recovered from a variety of processed foods where rosemary or sage were present (Ternes and Schwarz, 1995).

A number of assays for the determination of antioxidant activity have been developed ranging from the simple ß-carotene/linoleic acid agar diffusion technique of Araujo and Pratt (1985), Ascensão *et al.* (1998) to the spectrophotometric assay for TEARS (thiobarbituric acid reactive species) in the presence of inducers (Dorman *et al.,* 1995; Baratta *et al.,* 1998).

In 1996, Cuvelier *et al.* found eight sage *(Salvia officinalis)* extracts, originating from pilot-plant or commercial sources, had different antioxidative activities as measured by accelerated autoxidation of methyl linoleate. The extracts showed great variation in their HPLC profiles, and no correlation was apparent between their antioxidative efficiency and their composition. Data indicated that the most effective compounds were carnosol, rosmarinic acid, and carnosic acid, followed by caffeic acid, rosmanol, rosmadial, genkwanin and cirsimaritin.

Correlation studies between leaf senescence rates of watercress, *Petroselinum crispum* and *Salvia officinalis* and their oxidative defence systems were conducted with detached leaves under simulated shelf-storage conditions (Philosoph-Hadas *et al.,* 1994; Meir *et al.,* 1995). The relative order of leaf senescence rate, based on the rate of chlorophyll degradation and malondialdehyde accumulation, was watercress > parsley > sage. However, all three species showed high proteolysis rates from the

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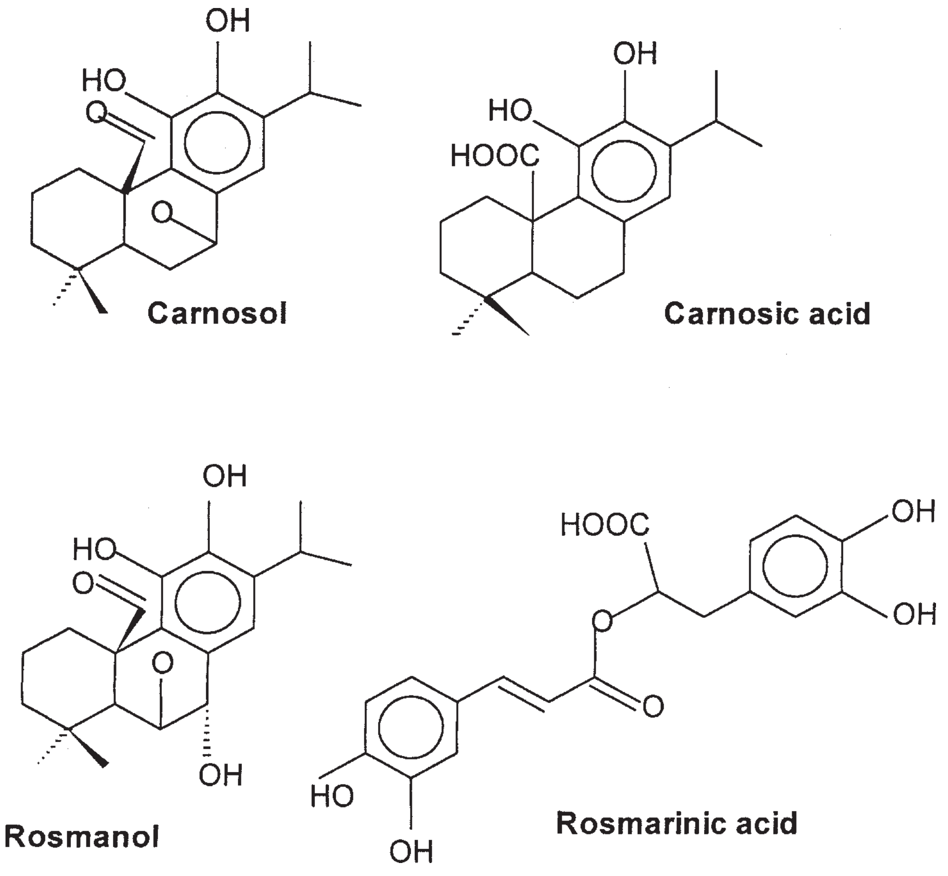

Figure 1 Major antioxidative compounds in *Salvia officinalis.*

first day of incubation. Of five oxidative defence systems examined in the three species, only total reducing capacity correlated well with the relative order of chlorophyll degradation and could therefore predict storage potential. The results indicate that each herb species has developed specific oxidative defence systems, which may also prevent rapid chlorophyll loss but do not affect proteolysis. It seems, therefore, that among the various components of the senescence syndrome, chlorophyll breakdown is closely linked to lipid oxidation, while proteolysis seems to proceed independently of these two senescence-associated processes.

A novel blend of seven herbal extracts, including sage, was developed for an improved immune response in human haemolysate causing a reduction in free radical damage and lower amounts of malondialdehyde. In addition, activities of all antioxidant enzymes increased, especially those of catalase and superoxide dismutase (Stajner *et al.,* 1997).

Rosmarinic acid is a natural antioxidant produced by cell suspension cultures of sage. Hippolyte *et al.* (1991, 1992) found that the growth and production of

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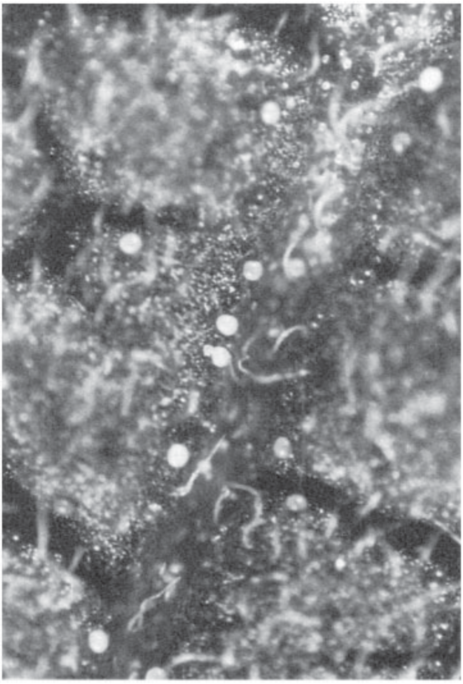
rosmarinic acid by sage cells was modified by the type of culture medium used. Rosmarinic acid production was increased 10-fold to attain 6.4gL^{-1} under optimal conditions. Investigation of cell growth kinetics showed that a change in the medium caused shifts in peaks of growth and rosmarinic acid production, and modifications of the cell metabolism. By changing the composition of the culture medium it was possible to manipulate rosmarinic acid production to coincide with cell growth or to begin only when growth had stopped.

One problem which does arise with using aromatic plant extracts as antioxidants in foodstuffs is the fact that they usually have a strong odour and bitter taste. However, in 1977 an effective antioxidant, in an odourless and tasteless form, was prepared from rosemary and sage. Its antioxidant activity was demonstrated in both animal and vegetable oils (Chang *et al.,* 1977). The use of supercritical carbon dioxide is a useful extraction technique which avoids the residue problems associated with solvent extraction. In 1995, the use of supercritical carbon dioxide was reported for the extraction of effective natural phenolic antioxidants from sage (Gerard *et al.,* 1995).

There is a real need for natural antioxidants at a time when the synthetic moieties currently in use are receiving considerable attention with respect to their safety and acceptability. Aromatic and medicinal plants such as members of the genus *Salvia* will provide such safer alternatives.



Photograph 1 Glandular hairs on leaf of *Salvia officinalis* (incident illumination).

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Photograph 2 Stalked glandular hair on leaf of *Salvia officinalis* (stained gentian violet/ eosin).

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13. THE ANTIHYPERTENSIVE PROPERTIES OF DAN-SHEN, THE ROOT OF *SALVTA MILTIORRHIZA*

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Salviae Miltiorrhizae Radix (*Salvia miltiorrhiza* BUNGE) is a galenical drug used to treat disturbances of the vascular system; it is widely employed in China to improve blood flow and dilate blood vessels (Wang *et al.,* 1978; Microcirculation Research Group, 1978; Onitsuka *et al.,* 1983; Chen, 1984). It has been reported that this drug contains naphthoquinone (phenanthrene quinone) derivatives such as tanshinone I, tanshinone II-A, tanshinone II-B, cryptotanshinone, isotanshinone I, isotanshinone II, isocryptotanshinone, tanshinonic acid, hydroxytanshinone and miltirone as active components (Kakisawa *et al.,* 1968; Inouye and Kakisawa, 1969; Kakisawa *et al.,* 1969; Shibata *et al.,* 1982).

In a previous study aimed at determining the beneficial effects of Salviae Miltiorrhizae Radix, we focused on changes in renal function parameters in rats with renal failure showing disturbance of the vascular system, and found that glomerular filtration rate, renal plasma flow and renal blood flow, which were decreased under the conditions of renal failure, were increased significantly after oral administration of Salviae Miltiorrhizae Radix (Chung *et al.,* 1987). In addition, we isolated the active constituent of this crude drug using renal function parameters as markers, and identified it as magnesium lithospermate B, a tetramer of caffeic acid, on the basis of data obtained from ^{13}C-NMR, IR, negative ion FAB-MS, ^{1}H-NMR and energy-dispersion X-ray analyses (Tanaka *et al.,* 1989; Yokozawa *et al.,* 1989a). The relative configuration of this substance was also determined. Our further investigations of the mechanisms of action of magnesium lithospermate B suggested that improved production and secretion of prostaglandin E_2 as a result of kallikrein activation was responsible for the vasodilation, increased blood flow and improved renal function (Yokozawa *et al.,* 1989b, 1990a, 1990b, 1991a).

Kallikrein, which probably initiates the reaction to magnesium lithospermate B, is a representative depressor peptide. It has been demonstrated by Laragh (1981) that this substance also contributes to the regulation of blood pressure through interaction with other vasoactive systems such as the renin-angiotensin-aldosterone, sympathetic nerve, vasopressin and prostaglandin systems through direct action on the cardiovascular system and through metabolism of water and sodium. In parti cular, attention has been focused on a decrease in the kinin-kallikrein system as a possible causative factor of essential hypertension.

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In this connection, the present study was carried out to investigate the effect on blood pressure of magnesium lithospermate B and other caffeic acid analogues isolated from Salviae Miltiorrhizae Radix. The levels of kallikrein, sodium and prostaglandin E_2 excretion, which exert influences on blood pressure, were also determined.

ADENINE-INDUCED RENAL HYPERTENSION

The most favourable type of antihypertensive drug would be one that is sufficiently effective without any combined therapy and having minimal side effects, while preventing or ameliorating disorders of organs including the kidney. From this viewpoint, ß-blockers, central a_2-stiraulants, angiotensin converting enzyme inhibitors and calcium antagonists are generally used clinically to treat renal hypertension (Williams, 1992). The experimental data reported by Tolins and Raij (1990) indicate that angiotensin converting enzyme inhibitors may be superior to calcium antagonists in halting the progression of renal dysfunction. However, the action of angiotensin converting enzyme inhibitors in decreasing the intraglomerular pressure can lead to deterioration of renal function, and therefore the influence of antihypertensive therapy on renal function is now attracting serious attention.

In rats with renal failure induced by adenine, it has been confirmed previously, both histologically and biochemically, that the renal failure progresses as the period of adenine-feeding is prolonged, and that blood pressure increases under the conditions of renal failure (Yokozawa *et al.,* 1986, 1987a, 1987b, 1989c; Yokozawa and Oura, 1987; Koeda *et al.,* 1988; Oura *et al,* 1991). Similar results were obtained in the present experiment. However, renally hypertensive rats given oral magnesium lithospermate B showed a significant decrease in systolic, mean and diastolic blood pressure. This effect became more marked as the administration period was extended and renal dysfunction advanced, as shown in Table 1. As has been reported previously (Yokozawa *et al.,* 1991b, 1993), it is apparent that magnesium lithospermate B improves renal function and promotes the excretion of uremic toxins, which accumulate in the body in parallel with the progression of renal failure. The anti-hypertensive effect seen in the present study is therefore attributable to the magnesium lithospermate B-induced improvement in renal hemodynamics. This is corroborated by our previously reported finding that magnesium lithospermate B promotes the activity of the kallikrein-prostaglandin system (Yokozawa *et al.,* 1989b, 1990a, 1990b, 1991a), which influences renal hemodynamics. We have also demonstrated in an experiment using sliced kidney specimens and isolated kidney microsomes that the action of magnesium lithospermate B is mediated by the kinin-prostaglandin system (Chung *et al.,* 1995).

Lithospermic acid B, which has the chemical structure of magnesium lithospermate B but lacks a magnesium salt, showed an action similar to that of magnesium lithospermate B, indicating that this action does not depend on the magnesium salt, but on lithospermic acid B, a tetramer of caffeic acid. However, in rats given caffeic acid trimer, having a lower molecular weight, there were no significant changes in systolic, mean and diastolic blood pressure. No significant

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Table 1 Effect of caffeic acid analogues on blood pressure in rats with adenine-induced renal failure.

Day	*Group*	*Systolic blood pressure (mmHg)*	*Mean blood pressure (mmHg)*	*Diastolic blood pressure (mmHg)*
6	Control	146.2 ± 3.1	116.5 ± 4.4	101.4 ± 6.0
	Magnesium lithospermate B	136.7 ± 3.3	109.5 ± 3.0	95.9 ± 4.3
	Lithospermic acid B	139.8 ± 2.8	112.5 ± 3.0	99.0 ± 3.3
	Lithospermic acid	143.3 ± 2.5	112.5 ± 3.6	104.7 ± 3.4
	Rosmarinic acid	141.3 ± 1.8	109.9 ± 1.5	97.6 ± 2.0
	Caffeic acid	149.7 ± 2.0	118.0 ± 2.6	102.0 ± 3.6
12	Control	156.2 ± 3.3	120.0 ± 6.1	101.7 ± 6.9
	Magnesium lithospermate B	143.9 ± 2.3[a]	109.1 ± 2.4	91.1 ± 3.0
	Lithospermic acid B	145.4 ± 2.6[a]	112.2 ± 2.5	92.5 ± 5.1
	Lithospermic acid	147.4 ± 4.2	108.7 ± 3.4	92.3 ± 4.5
	Rosmarinic acid	149.0 ± 4.3	115.2 ± 3.9	90.7 ± 6.2
	Caffeic acid	153.2 ± 3.1	120.6 ± 3.0	103.6 ± 3.6
18	Control	175.4 ± 3.7	136.3 ± 2.9	119.9 ± 3.3
	Magnesium lithospermate B	156.6 ± 2.0[b]	120.3 ± 4.0[b]	98.2 ± 4.4[b]
	Lithospermic acid B	162.0 ± 4.0[a]	127.7 ± 4.6	110.0 ± 4.4
	Lithospermic acid	163.9 ± 4.7	131.1 ± 4.2	116.9 ± 4.1
	Rosmarinic acid	166.1 ± 4.3	133.6 ± 3.3	120.6 ± 6.7
	Caffeic acid	165.9 ± 2.6	129.9 ± 2.0	116.4 ± 2.2
24	Control	183.5 ± 3.4	145.7 ± 6.3	127.3 ± 5.9
	Magnesium lithospermate B	157.2 ± 3.3[c]	122.7 ± 2.8[b]	104.7 ± 3.3[b]
	Lithospermic acid B	167.3 ± 3.1[b]	129.0 ± 5.8	115.2 ± 4.7
	Lithospermic acid	175.4 ± 4.1	134.7 ± 5.1	118.9 ± 6.1
	Rosmarinic acid	180.3 ± 3.6	140.1 ± 5.7	125.6 ± 6.0
	Caffeic acid	174.9 ± 3.0	142.6 ± 3.2	124.3 ± 3.3

Significantly different from the control value: [a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$.

decrease in blood pressure was also observed upon administration of analogues with even lower molecular weights, i.e., caffeic acid dimer and caffeic acid (Table 1 and Fig. 1). Thus, the manifestation of drug action shows a structure-specific pattern.

The kallikrein-kinin system is a representative depressor peptide system in the body. This system causes smooth muscle to relax, leading to a decrease in blood pressure, and acts on the kidney to promote excretion of water and sodium. It is believed that this system is involved in blood pressure regulation in cooperation with the pressor renin-angiotensin-aldosterone system, sympathetic nerve system and the depressor prostaglandin system (Abe *et al.,* 1978). Since Elliot and Nuzum first reported in 1934 that the urinary excretion of kallikrein was decreased in patients with hypertension, various researchers including Margolius *et al.* (1971), Miyashita (1971) and Seino *et al.* (1975) have observed decreased urinary excretion of kallikrein in hypertensive patients. The present study also found a marked decrease

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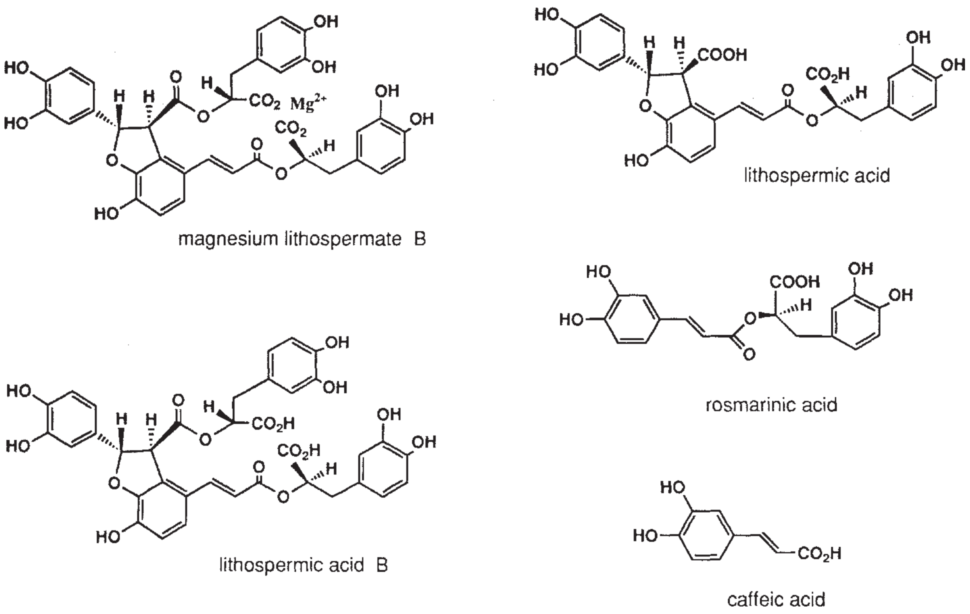

Figure 1 Chemical structures of caffeic acid analogues from *Salviae Miltiorrhizae* Radix.

in excretion of kallikrein along with the increase in blood pressure. In contrast, magnesium lithospermate B and lithospermic acid B, both having an antihypertensive action, induced a significant increase in kallikrein excretion, as shown in Table 2. Since magnesium lithospermate B has previously been proved to have no effects on the pressor renin-angiotensin-aldosterone system (Yokozawa *et al.,* 1990b), it is strongly suspected that this depressor peptide is involved in the decrease of blood pressure induced by magnesium lithospermate B and lithospermic acid B. It has been reported by Omata *et al.* (1982) that the level of urinary excretion of kallikrein reflects the amount of kallikrein produced in the kidney. The promotion of kallikrein excretion by the active components of Salviae Miltiorrhizae Radix is therefore suggested to result from a direct action on the kidney. Rats given magnesium lithospermate B or lithospermic acid B also showed a significant increase in sodium excretion in parallel with the variation in kallikrein (Table 2). This also corroborates a probable direct action on the kidney.

SODIUM-INDUCED HYPERTENSION AND RENAL FAILURE

Arterial hypertension is a very common finding in chronic renal failure. Studies of the mechanism underlying the appearance of high blood pressure have revealed the existence of an increased extracellular fluid volume that correlates with systemic pressure and the presence of subtle abnormalities in the sodium-renin feedback

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Table 2 Effect of caffeic acid analogues on urinary excretions of kallikrein and sodium in rats with adenine-induced renal failure.

Day	*Group*	*Kallikrein (mU/24 h)*	*Na (mM/24 h)*
12	Control	23.39 ± 1.52	1.67 ± 0.08
	Magnesium lithospermate B	38.50 ± 5.83[a]	1.86 ± 0.11
	Lithospermic acid B	31.06 ± 3.25[a]	1.79 ± 0.12
	Lithospermic acid	26.18 ± 1.70	1.71 ± 0.09
	Rosmarinic acid	26.20 ± 3.67	1.64 ± 0.12
	Caffeic acid	26.22 ± 3.45	1.67 ± 0.19
24	Control	13.77 ± 1.50	0.89 ± 0.06
	Magnesium lithospermate B	21.59 ± 0.92[b]	1.15 ± 0.04[b]
	Lithospermic acid B	17.90 ± 0.89[a]	1.07 ± 0.05[a]
	Lithospermic acid	15.00 ± 2.01	0.95 ± 0.09
	Rosmarinic acid	14.87 ± 1.87	0.90 ± 0.09
	Caffeic acid	13.96 ± 1.58	0.91 ± 0.10

Significantly different from the control value: $^{a}p < 0.05$, $^{b}p < 0.01$.

mechanism. It is also well known that renal vasodepressor substances such as kallikrein, kinin and prostaglandins may play a physiological role in sodium excre tion, and that hypertension may result from a deficiency of the renal depressor system (Coleman *et al.,* 1975). In a previous study, we demonstrated that a reduced level of urinary sodium excretion in rats with adenine-induced renal failure, which was significantly correlated with the reduced level of urinary prostaglandin E_2 excretion (it has been shown by Frölich *et al.* (1975) that prostaglandin E_2 in urine is mostly derived from the kidney), may play an important role in the development of hypertension (Yokozawa *et al.,* 1991a). Similar results were obtained in the present experiment using high-salt-induced hypertension with renal failure. It was also found that administration of magnesium lithospermate B produced a significant reduction in blood pressure, accompanied by a significant increase of urinary sodium excretion, as shown in Tables 3 and 4.

Yoshida *et al.* (1986) demonstrated that in the early phase of two-kidney one-clip renovascular hypertension in animals, the renin-angiotensin-aldosterone system contributes to the development of hypertension, and that blood pressure shows no significant change after sodium loading or restriction. On the other hand, in the chronic phase of this model, the role of the renin-angiotensin-aldosterone system is no longer obvious, and changes in sodium metabolism might play a role in the maintenance of hypertension. Because we did not measure parameters of the reninangiotensin-aldosterone system in the present study, it was difficult to confirm any effect of magnesium lithospermate B on this system. However, in a previous study, we observed that magnesium lithospermate B significantly increased urinary electrolytes and the excretion of prostaglandin E_2 and kallikrein in normal rats, whereas no significant changes were observed in the renin-angiotensin-aldosterone

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Table 3 Effect of magnesium lithospermate B on blood pressure in rats with sodium-induced hypertension and renal failure.

Day	*Group*	*Dose (mg/kg B.W./day)*	*Systolic blood pressure (mmHg)*	*Mean blood pressure (mmHg)*	*Diastolic blood pressure (mmHg)*
12	Control	–	192.9 ± 7.6	150.5 ± 6.6	129.1 ± 6.4
	Magnesium lithospermate B	5	181.9 ± 6.1	131.4 ± 5.3[a]	106.0 ± 6.3[a]
	Magnesium lithospermate B	10	170.4 ± 5.2[a]	130.4 ± 3.9[a]	110.3 ± 3.8[a]
24	Control	–	208.7 ± 9.4	167.4 ± 7.8	146.5 ± 7.3
	Magnesium lithospermate B	5	190.3 ± 6.1	144.8 ± 6.5[a]	121.8 ± 7.3[a]
	Magnesium lithospermate B	10	184.7 ± 5.2[a]	147.0 ± 3.9[a]	128.0 ± 4.6[a]

Significantly different from the control value: $^{a}p < 0.05$.

Table 4 Effect of magnesium lithospermate B on urine volume and urinary sodium excretion in rats with sodium-induced hypertension and renal failure.

Day	*Group*	*Dose (mg/kg B.W./day)*	*Urine volume (ml/24 h)*	*Na (mM/24 h)*
12	Control	–	94.4 ± 4.1	9.09 ± 0.47
	Magnesium lithospermate B	5	104.7 ± 7.1	11.35 ± 0.57[b]
	Magnesium lithospermate B	10	102.1 ± 4.2	11.88 ± 0.50[c]
24	Control	–	59.5 ± 7.6	5.32 ± 0.64
	Magnesium lithospermate B	5	58.9 ± 7.3	5.51 ± 0.59
	Magnesium lithospermate B	10	91.7 ± 7.7[b]	6.96 ± 0.25[a]

Significantly different from the control value: $ap < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

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system (Yokozawa *et al.,* 1990b). Therefore, it seems that magnesium lithospermate B does not act to regulate blood pressure through the renin-angiotensin-aldosterone system.

The prostaglandins together with the kallikrein-kinin system make up a major vasodepressor system that opposes the pressor effect of the renin-angiotensin-aldosterone system and is suggested to participate in the control of blood pressure (McGiff 1980; Dunn and Hood 1977). It has been shown that prostaglandin E_2 not only increases renal blood flow by dilating renal blood vessels, but also produces relaxation of mesangial cells. Although it is still questionable whether urinary excretion of prostaglandin E_2 may directly indicate the state of circulating or local vascular levels of these vasodilatory substances, our results shown in Table 5 seem to support the hypothesis that prostaglandin E_2 in magnesium lithospermate B-treated rats may contribute to the blood pressure-lowering effect. It is possible that increased synthesis of prostaglandin E_2 may be one of the factors responsible for the increased excretion of sodium. In this regard, Haas *et al.* (1988) and Romero and Knox (1988) recently proposed that changes in renal prostaglandin levels may mediate pressure-natriuresis by inhibiting reabsorption in the proximal tubule of deep nephrons, and that renal prostaglandins might counteract increases in renal vascular resistance and contribute to the maintenance of renal function.

Kunze and Vogt (1971) and Vargaftig and Hai (1972) have demonstrated the following mechanism of prostaglandin release: kallikrein activates phospholipase A_2, resulting in enhanced production of arachidonic acid and prostaglandin synthesis. On the other hand, in an experiment using perfused rat kidney, Roblero *et al.* (1976) have shown that kallikrein in urine is derived from the kidney. It has also been reported that this substance is produced in conjugated uriniferous tubules (Orstavik *et al.,* 1976). In the present study, the urinary excretion of kallikrein in rats given magnesium lithospermate B increased as the dose of the compound was stepped up. Although the degree of the increase in kallikrein was not consistent with that in prostaglandin E_2, the pattern of the increase was similar. This clearly indicates that magnesium lithospermate B activates the kallikrein-prostaglandin system.

Margolius *et al.* (1974) found that urinary kallikrein excretion was reduced in many patients with essential hypertension, and demonstrated that there was a blunted response to dietary sodium restriction in comparison with normal subjects. It has also been observed that urinary kallikrein was uniformly reduced in renal hypertensive rats (Margolius *et al.,* 1972). In dogs with chronic unilateral renal artery stenosis, Keiser *et al.* (1976) found a marked reduction of kallikrein excretion from the stenotic kidney, and this excretion rate was correlated well with the degree of reduction in renal blood flow. In the present study, urinary excretion of kallikrein was significantly increased by administration of magnesium lithospermate B. These data, taken together with the preceding results on urinary sodium and prostaglandin E_2 excretion, suggest that magnesium lithospermate B may ameliorate the development of hypertension by improving the renal circulatory state in rats with sodium-induced hypertension and renal failure. However, Guimaraes *et al.* (1986) have recently shown that in the perfused rat kidney, bradykinin and lysylbradykinin are rapidly converted to the corresponding des-arginine compounds, and that these

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Table 5 Effect of magnesium lithospermate B on urinary excretion of PGE_2 and kallikrein in rats with sodium-induced hypertension and renal failure.

Day	*Group*	*Dose (mg/kg B.W./day)*	*PGE_2 (ng/24 h)*	*Kallikrein (mU/24 h)*
12	Control	–	27.88 ± 2.34	24.12 ± 2.69
	Magnesium lithospermate B	5	42.85 ± 4.04[b]	34.93 ± 4.31[a]
	Magnesium lithospermate B	10	45.27 ± 4.72[b]	40.11 ± 4.92[b]
24	Control	–	6.45 ± 1.14	16.59 ± 2.20
	Magnesium lithospermate B	5	7.47 ± 0.82	36.21 ± 1.13[a]
	Magnesium lithospermate B	10	15.72 ± 4.46[a]	44.61 ± 8.92[b]

Significantly different from the control value: $^{a}p < 0.05$, $^{b}p < 0.01$.

Table 6 Effect of magnesium lithospermate B on blood pressure in SHR.

Day	*Group*	*Dose (mg/kg B.W./day)*	*Systolic blood pressure (mmHg)*	*Mean blood pressure (mmHg)*	*Diastolic blood pressure (mmHg)*
12	Control	–	223.7 ± 4.0	173.0 ± 3.2	147.4 ± 3.2
	Magnesium lithospermate B	5	212.6 ± 6.9	164.3 ± 4.1	140.0 ± 3.5
	Magnesium lithospermate B	10	206.7 ± 6.0[a]	158.6 ± 4.7[a]	134.3 ± 4.3[a]
24	Control	–	230.1 ± 5.4	178.1 ± 4.1	151.8 ± 4.5
	Magnesium lithospermate B	5	206.0 ± 5.3	157.0 ± 4.6	132.3 ± 5.3[a]
	Magnesium lithospermate B	10	206.3 ± 6.4[b]	154.7 ± 5.9[b]	127.0 ± 6.1[b]

Significantly different from the control value: $^{a}p < 0.05$, ^{b}p> < 0.01.

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then lose their vasodilator activity and become vasoconstrictors by acting on the B1 receptors. Also, it has been reported that infusion of angiotensin in the rat reduces the excretion of kallikrein (Mills *et al.,* 1989). One would then not expect an increase in kallikrein to lower blood pressure unless it were acting only as a stimulator of phospholipase.

SPONTANEOUSLY HYPERTENSIVE RATS

The present study was also carried out to investigate the effect on blood pressure of magnesium lithospermate B in spontaneously hypertensive rats, a widely used model of essential hypertension. Oral administration of magnesium lithospermate B in spontaneously hypertensive rats resulted in a significant decrease of systolic, mean and diastolic blood pressures. The longer the administration period, the more marked this effect became, as shown in Table 6. Administration of magnesium lithospermate B also significantly increased the low urinary kallikrein level in spontaneously hypertensive rats, with a parallel increase in the excretion of prostaglandin E_2 and sodium (Table 7). These data suggest that magnesium lithospermate B may ameliorate the development of hypertension by improving the renal circulatory state. Stokes and Kokko (1977) found in a study of isolated perfused tubules that prostaglandin E_2 caused direct inhibition of sodium reabsorption in the tubule. Ruilope *et al.* (1982) demonstrated the protective role of renal prostaglandin E_2 in the maintenance of hypertension. Thus, it appears that the depressor effect of magnesium lithospermate B results from direct action in the kidney. This idea was also supported by the blood pressure-lowering action of magnesium lithospermate B in rats with renal failure and sodium-induced hypertension.

Since antihypertensive drugs are usually taken for a prolonged period, their proven safety is essential. The results of a previous study on the acute toxicity of oral magnesium lithospermate B in terms of LD_{50} determined by the up and down method showed high safety of this substance (>3000 mg/kg in 6-week-old male ddy mice weighing 31–35 g).

CONCLUSION

The antihypertensive effect of magnesium lithospermate B isolated from Salviae Miltiorrhizae Radix was evaluated using rats with adenine-induced renal failure and hypertension, rats with sodium-induced hypertension and renal failure and spontaneously hypertensive rats. Oral administration of magnesium lithospermate B lowered the systolic, mean and diastolic blood pressures in hypertensive rats, in comparison with the progressive hypertension observed in untreated control animals. Urinary excretion of sodium, kallikrein and prostaglandin E_2 was increased significantly in rats given magnesium lithospermate B. These data suggest that magnesium lithospermate B is useful for treatment of hypertension.

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Table 7 Effect of magnesium lithospermate B on urine volume and urinary excretion of PGE_2 and kallikrein in SHR.

Day	*Group*	*Dose (mg/kg B.W./day)*	*Urine volume (ml/24 h)*	*Na (mM/24 h)*	*PGE_2 (ng/24 h)*	*Kallikrein (U/24 h)*
12	Control	–	17.9 ± 1.9	2.49 ± 0.01	22.06 ± 1.80	1.13 ± 0.04
	Magnesium lithospermate B	5	18.3 ± 2.1	2.66 ± 0.01^{c}	25.37 ± 1.37	1.37 ± 0.06^{b}
	Magnesium lithospermate B	10	19.0 ± 1.0	2.85 ± 0.01^{c}	27.80 ± 1.41^{b}	1.62 ± 0.09^{c}
24	Control	–	21.4 ± 3.9	2.56 ± 0.01	23.26 ± 1.40	1.30 ± 0.19
	Magnesium lithospermate B	5	18.5 ± 1.7	2.73 ± 0.01^{c}	30.24 ± 2.04^{a}	1.76 ± 0.09
	Magnesium lithospermate B	10	22.4 ± 1.4	2.84 ± 0.01^{c}	34.89 ± 1.69^{b}	2.26 ± 0.16^{b}

Significantly different from the control value: $^{a}p < 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$.

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14. WHY SAGE MAY BE A WISE REMEDY: EFFECTS OF *SALVIA* ON THE NERVOUS SYSTEM

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> "Sage will retard that rapid progress of decay that treads upon our heels so fast in latter years of life, will preserve faculties and memory more valuable to the rational mind than life itself" (John Hill 1756).

The human brain is the most complex system known in the universe. There are over 10 billion nerve cells or neurons, each of which connects to 10 thousand others to form the systems and pathways that govern all our mental functions—mood, memory, sleep, dreaming for example and consciousness itself (Figure 1). Each neuron contains a particular transmitter—the chemical signal that is released when the neurons fire interacting with specific receptors so allowing the neurons to communicate with each other (Figure 2). There are over 50 of these chemical signals, which include glutamate, γ-aminobutyric acid (GABA), acetylcholine, noradrenaline, dopamine and serotonin. The glutamate and GABA signals are the principal excitatory and inhibitory transmitters which "drive" the system. Superimposed on this framework are the modulatory transmitters which govern the mode of the system. Acetylcholine for example is concerned with attention, memory and dreaming, noradrenaline is concerned with arousal and responding to threatening or exciting stimuli, dopamine is concerned with drive, motivation and pleasure, and serotonin or 5-HT is thought to be concerned with mood.

Many of the drugs used today for the treatment of disorders of the mind/brain (such as depression, insomnia, psychosis, memory loss, anxiety, epilepsy and movement disorders like Parkinson's disease) work by altering these chemical signalling pathways. Antidepressants for example work by increasing the level of serotonin, anxiolytics by promoting the action of the inhibitory transmitter GABA, anti-epileptics/anticonvulsants by supressing the action of glutamate, antipsychotics by inhibiting the action of dopamine, anti-Parkinsonian drugs by promoting the action dopamine and the more recently developed, memory enhancing/anti-dementia drugs that work by promoting the action of acetylcholine. Long before these complex brain mechanisms were understood, plant medicines have been used

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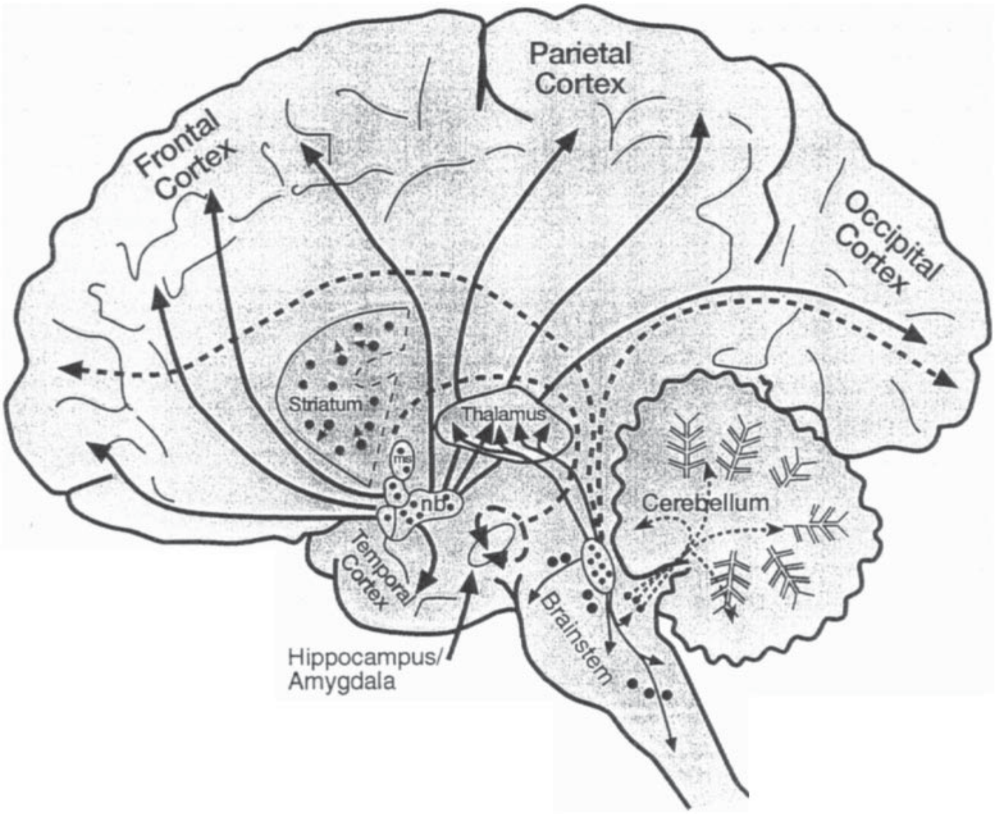

Figure 1 Diagram of the human brain showing an example of one of the neutransmitter pathways using acetylcholine which is involved in controlling attention and memory.

to treat these various CNS conditions, for example belladonna for Parkinson's disease, the opium poppy for analgesia and sleep, valerian for anxiety/stress and more recently St John's Wort for depression.

In the literature numerous other examples can be found of extracts and isolated constituents that have *in vitro* and *in vivo* effects on the CNS. For example, more than 50 herbs with CNS activities are the subject of monographs in the Chinese Pharmacopoeia (Zhu *et al., 1996*). Among the many prescriptions used in Chinese herbal medicine a significant number, including *Salvia* species, are used for the treatment of mental disorders such as depression, epilepsy, brain hypoxia and senile amnesia (Cho *et al.,* 1994; Chung *et al.,* 1994; Dhawan, 1994; Okugawa *et al.,* 1996; Su *et al.,* 1994). Many of the active principles from these medicinal plants that have been tested in relevant pharmacological models, act on CNS receptors including GABA, dopamine, muscarinic and benzodiazepine (Zhu *et al.,* 1996 and 1997). Amongst these plants with beneficial effects on the brain/CNS, *Salvia* features as a genus with widespread benefits for a range of disorders.

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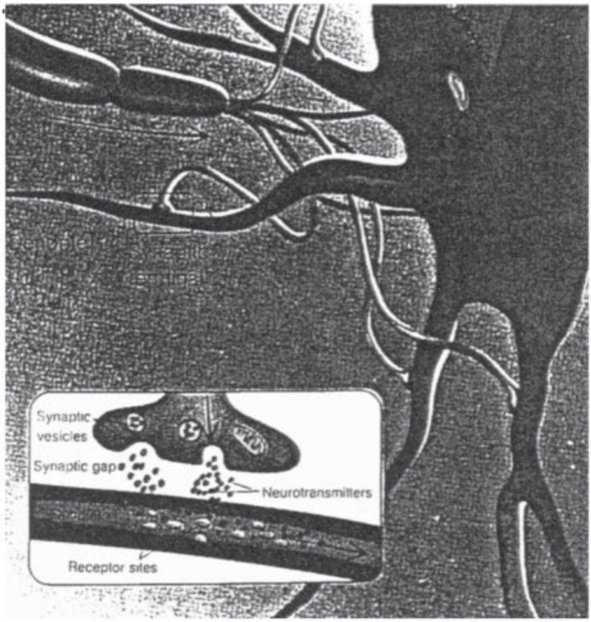

Figure 2 Picture of a nerve cell (neuron) with an inset showing the synaptic junction between the axon and dendrite and the release of transmitters from the axon interacting with receptors on the dendrite (adapted from 'The Brain', 1984, Edited by R.B. Pinchot).

SALVIA AND THE CNS

'Salvia' comes from the Latin *salvare* meaning "to be saved "and the genus has been valued since ancient times for its medicinal properties. *Salvia* (with over 700 species) is the largest genus in the Labiatae (Mint) family and, although not all have been researched pharmacologically, a number have actions on the CNS. The genus was known to the Egyptians as "anusi", to the Greeks as "elelisphakon" to the Romans as "herb sacra" the "salve"(saviour) and to Spanish and Moroccan Arab herbalists as "salima" or "asphacus" (Rivera *et al.,* 1994). There may be confusion in the older (herbal) literature as to which particular *Salvia* species is denoted by each of these names. Thus assignment of medicinal value to a particular species is not always clear-cut when its ancient use is mentioned.

Salvia species have been widely used since the Middle Ages to relieve various disorders including constipation, colds, fevers, cholera, liver problems, epilepsy and nervous disorders. An English proverb writes "He that would live for aye, Must eat Sage in May!" and the English herbalist John Gerard (1597) said "No man need doubt of the wholesomeness of Sage Ale, being brewed as it should be, with Sage, Scabious, Betony, Spikenard, Squinath and Fennel Seeds" (Grieve, 1980; Tyler, 1993). The genus features prominently in the pharmacopoeias of many countries throughout the world, particularly in China where 3 times the amount of their best tea was traded for European sage tea. European sage (for example *Salvia officinalis*

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and S. lavandulaefolia) and Chinese sage (*S. miltiorrhiza* Bunge.) have been widely investigated phytochemically and a vast array of biological activities have been identified, many of which from *S. miltiorrhiza* are relevant to CNS disorders.

Anxiolytic and Sedative Properties

Anxiolytic drugs are used to treat the symptoms of anxiety and are usually associated with sedation. One of their main effects involves increasing the synaptic inhibitory action of GABA and the most common class of drugs known are the benzodiazepines which act at a particular site on the $GABA_A$ receptor. There are a number of plants commonly used for their anxiolytic (in addition to hypnotic, carminative and antispasmodic) properties including feverfew *(Tanacetum parthenium)* and valerian *(Valeriana officinalis)* that affect $GABA_A$ receptors (Öztürk *et al.,* 1996).

A number of *Salvia* species are used as sedatives throughout the world. For example, *S. guaranitica* is used by Amazonian Indians as a sedative and hypnotic (inducing sleep); *in vitro* studies have shown the constituents cirsiliol and a caffeic acid ethyl ester to be competitive benzodiazepine (GABA) receptor ligands (Marder *et al.,* 1996). *S. compositus* has also shown CNS depressant action on electrical activities of the cerebral cortex (Fan *et al.,* 1979). The two diterpenoids, carnosic acid and carnosol, present in many *Salvia* species including *S. apiana* and *S.officinalis* have in addition to their antioxidant effects, inhibitory action on GABA receptors by inhibiting ligand binding to the chloride channels of the receptor (Dentali and Hoffman, 1990; Rutherford *et al.,* 1992).

There are several reports of *S. miltiorrhiza* root (Danshen) being analgesic and sedative. A reduction in the spontaneous activity of mice, and an increase in the duration of the hypnoticaction induced by chloral hydrate and barbiturates in the presence of *S. miltiorrhiza* root, have been observed (Huang, 1993; Chang *et al.,* 1986). In rabbits, *S. miltiorrhiza* root injection has demonstrated a reduction of spontaneous electrical activity of the cerebral cortex, an increase of late action potential threshold following repeated stimulation, and may potentiate an induced action potential of sensory stimulation (Chang *et al.,* 1986). Further investigation has established a structure-activity relationship of miltirone, a quinone isolated from *S. miltiorrhiza* root, as an active central benzodiazepine (GABA) receptor ligand (Chang *et al.,* 1991). Miltirone and perhaps other quinones from *S. miltiorrhiza* may explain the tranquillising effects observed, and may aid the development of a novel class of anxiolytic agents.

Related to anxiolytic/sedative effects which are thought to be due (principally) to action on the GABA receptors are anticonvulsant effects which are mediated by the same transmitter receptors. There are reports of anti-epileptic properties of *Salvia* species particularly of *S. officinalis* and the monoterpenoid linalool, present in the essential oils of various species, has anticonvulsant action via inhibition of glutamate binding, the main excitatory neurotransmitter in the CNS (Elisabetsk y, 1995; Puleo, 1978). Linalool is also a constituent of many European herbs in the Labiatae family such as *Melissa officinalis* (well known for its calming properties) and is present in

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Aeolanthus suaveolens that is used as an anticonvulsant in the Brazilian Amazon (Shultes, 1993).

Memory-Enhancing Properties

Memory declines as a natural part of the ageing process particularly in the latter years of life and in dementia of old age, for example, of the Alzheimer Type (Alzheimer's Disease). Alzheimer's disease (AD) is a common neurodegenerative disease that affects the elderly population and the primary symptom is a loss of memory. A consistent neuropathological finding associated with this memory loss is a cholinergic deficit, evident from a decrease in the cholinergic enzyme choline acetyltransferase which is involved in the synthesis of the neurotransmitter acetyl choline (ACh). The primary treatment, aimed at enhancing cholinergic activity (and therefore memory), inhibits the enzyme acetylcholinesterase (AChE) which breaks down ACh and so increases overall levels of ACh available for transmission. Thus synthetic drugs inhibiting AChE, for example tacrine, donepezil and rivastigmine, are used to treat memory loss associated with AD.

Many plants contain anticholinesterases and physostigmine from the Calabar bean *(Physostima venenosum)* was one of the first to be isolated. Interestingly there are also constituents with cholinesterase activity though their function is unknown (Gupta and Gupta, 1997). Further examples of plant-derived anticholinesterases are huperzine A from the moss *Huperzia serata* (Tang, 1994) and galantamine (currently in stage III clinical trials for AD) discovered in the bulbs of snowdrops *(Galanthus nevalis)* and later daffodils (*Narcissus* spp.) and also present in the Chinese herb *Lycorus radiata* (Bores *et al.,* 1996).

Plants have been used to treat memory-related disorders for centuries, for example *Huperzia serata* and *Ginkgo biloba* have been used in Chinese medicine and *Salvia officinalis and S. lavandulaefolia* in Europe. Plants are used by native Amazonian Indians to treat their unusually elderly population who suffer conditions that "superficially appear to resemble AD or related mental problems" (Shultes, 1993). A further example of a plant used to enhance memory is *Withania somnifera* (Indian Ginseng). It is used in Ayurvedic medicine to "attenuate cerebral functional deficits, including amnesia, in geriatric patients". Defined extracts (sitoindosides VII-X and withaferin-A) reversed both a cognitive deficit and a reduction in cholinergic markers in a rat model of cholinergic dysfunction (Bhattacharya *et al.,* 1995; Schliebs *et al.,* 1997). The ancient Chinese Maidenhair tree, *Gingko biloba* Linn, is reported to "stabilise and improve the cognitive performance and social functioning of demented patients" and has reached clinical trials for use in the treatment of AD (Le Bars *et al.,* 1997; Kanowski *et al.,* 1996). It has been held sacred for its health-promoting properties for thousands of years in China and leaf extracts are highly popular in the U.S and are marketed to "enhance cerebral blood flow and improve memory" and its toxic effect is markedly low (Reuter, 1996). A *Gingko biloba* extract has beneficial effects on blood flow (Smith *et al.,* 1996) and has other pharmacological actions of relevance to AD therapy (Gräsel and Reuter, 1998; Reuter, 1996; Rodriguez *et al.,* 1993; Taylor, 1986).

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In Europe the use of medicinal plants has been, in the recent past, minimal compared to Asia mainly because of the advances in synthetic chemistry. Historically however, reviewing the literature (going back 400 years) herbs have been used for memory enhancement. Balm *(Melissa officinalis),* rosemary *(Rosmarinus offidnalis)* and *Salvia* spp. are documented as "strengthening the brain" or enhancing memory. John Hill (1751) said *M. officinalis* was "good for disorder of the head" and Cunningham (1993) reported that "a chaplet of rosemary, worn, aids the memory" and Greek students were said to wear garlands of this herb during exams.

The English herbalist John Gerard in 1597 said sage (not specifying the species) was "singular good for the head and brain" and with Nicolas Culpeper (in 1652) and the Greeks, said sage was "...excellent good use to help the memory by warming and quickening the senses" (Crellin, 1990; Ryman, 1991). *Salvia* tea is noted by Grieve (1980) as a "highly serviceable stimulant tonic in debility of the nervous system". *Salvia* is also used in Ayurvedic medicine where it is said "to clear emotional obstructions from the mind and for promoting calmness and clarity" (McIntyre, 1996).

A recent substantiation of anecdotal reports of the medicinal value of *Salvia* species for memory-enhancement is the anticholinesterase activity of essential oils and herb extracts of European *Salvia* (*S. officinalis* and *S. lavandulaefolia*) in human brain tissue (post-mortem) (Perry *et al.,* 1996). This could account, at least in part, for its memory enhancing reputation. *S. lavandulaefolia* lacks thujone—a toxic component of *S. officinalis* (commonly known to be anthelmintic, psychedelic and a uterine stimulant) so *S. lavandulaefolia* may be a more suitable *Salvia* species to investigate for memory enhancement. *S. lavandulaefolia* is approved by the FDA for food use and was given GRAS status by the FEMA in 1965 (Opdyke, 1976; Tisserand and Balacs, 1995).

Chromatographic fractionation of *S. lavandulaefolia* essential oil yielded 25 fractions, one of which inhibited human erythrocyte AChE (Perry *et al.,* 1997) and the compounds responsible were found to be monoterpenoids. There are previous

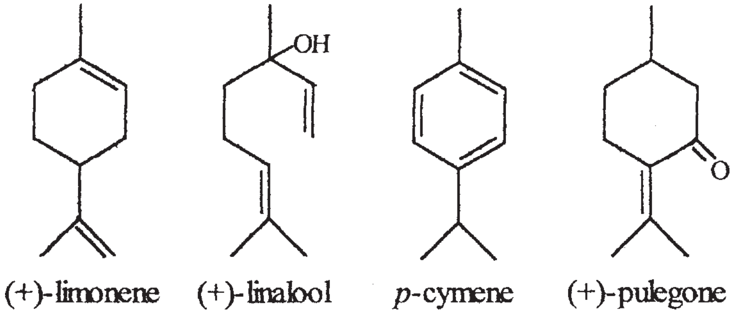

Figure 3 Examples of monoterpernoids that inhibit the enzyme acetylcholinesterase and therefore increase the amount of acetylcholine available for transmission at the cholinergic synapse. Limonene, linalool and *p*-cymene are present in many *Salvia* species including *S. lavandulaefolia and S. officinalis.*

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reports that several terpenoids, some of which occur in *Salvia,* inhibit AChE at relatively high (10^{-3}–10^{-4} M) concentrations, for example, limonene, linalool and *p*-cymene (Figure 3) and some may act synergistically (Gracza, 1985; Grundy and Still, 1985; Miyazawa *et al.,* 1997+1998; Ryan and Byrne, 1988).

In vivo oral administration of the essential oil to normal aged rats resulted in inhibition of AChE in select brain areas (Perry *et al.,* 1998). At the lower dose there was a significant decrease in AChE activity in the striatum but not the hippocampus or cortex of the *S. lavandulaefolia*-tteated compared to the control group. At the higher dose there was a significant decrease in AChE activity in the striatum and the hippocampus, but not the cortex, of the group treated with *S. lavandulaefolia* essential oil compared to the control group. The *in vivo* inhibition of AChE seen in the striatum may be of relevance to AD as this part of the brain is thought to participate in emotional and motivational behaviour and AD patients experience behavioural dysfunction (Selden *et al.,* 1994). One of the primary symptoms of AD is short-term memory loss and the hippocampus, that is severely affected in AD, plays a major role in short term memory. Thus the tendency seen here for *S. lavandulaefolia* essential oil to inhibit striatal and hippocampal AChE *in vivo* (thereby increase the presence of acetylcholine and thus cholinergic transmission) would be of significance to improve cognitive function particularly in AD. Also since the striatum is a major site of functional pathology in Parkinson's disease, in which there is a loss of dopa-minergic input, targeting this area may be important in Parkinson's disease treatment. The results of this *in vivo* study suggest that *S. lavandulaefolia* essential oil, following oral administration reaches the brain, that is crosses the blood-brain and gastro-intestinal barrier and may therefore be of therapeutic relevance.

Salvia also has reported antioxidant (Wong *et al.,* 1995; Cuvelier, 1996), oestrogenic (Tyler, 1993) and anti-inflammatory properties (Tyler, 1993; Bartram, 1995) (see below) and these actions are now considered valuable in AD therapy (Birge, 1997; Mortel and Meyer, 1995). The memory-enhancing reputation of *Salvia* species may thus be due to a combination of actions.

It is interesting to consider why plants produce cholinergic (CNS) active constituents. Plants have evolved a broad spectrum of chemical defense against a diverse group of predators and one mode of action stems from targeting the nervous system. Anticholinesterases are used by plants to interfere with (cholinergic) nervous transmission, thus paralysing and killing the insect. Many plant-derived cholinergic agents (for example, physostigmine, huperzine and galanthamine) are alkaloids, which are the most potent of all plant constituents and are associated with the toxicity of the plant. At high dose levels these agents are lethal to humans, however at lower levels their actions are therapeutic. Of the groups of secondary metabolites, the terpenoids are among those acting as plant defense compounds and they are the main constituents of the *Salvia* genus. It is of interest that the terpenoids are not harmful to all insects and indeed many are used in chemical recognition for example, as sex pheromones. This association of plant-insect co-evolution may lead to the discovery of active cholinergic compounds of a different nature and may lead to therapeutics with fewer side effects. Monoterpenoids present in *S. officinalis* and *S. lavandulaefolia* have *in vitro* anticholinesterase activity (Perry *et al.,* 1996) and *in*

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vivo monoterpenoids paralyse and kill insects (Ryan and Byrne, 1988) and yet most are considered non-toxic to humans.

Thus several plants have been investigated for their memory enhancing activity and have yielded compounds which may be of clinical relevance in future AD management. Although many plants exist that could possess memory enhancing properties, *Salvia* spp. are of interest because *S. officinalis* is a common culinary herb and *S. lavandulaefolia* is widely available and used all over world in the cosmetic and flavouring industry and can therefore be assumed to be relatively safe.

Antioxidant Activity

Many diseases of the nervous and non-nervous systems involve free radical damage due to excess reactive oxygen species. Cellular damage due to lipid, protein and DNA peroxidation is associated with a number of disorders. Of relevance here is its contribution to neuronal cell damage in neurodegenerative (CNS) disorders and ischaemia as well as the general ageing process. Free radical damage has been described in the pathological changes which occur in AD (Lyras *et al.,* 1997) and limiting free radical damage by antioxidants is considered to be an important thera peutic strategy in CNS disorders.

Common to many *Salvia* species, including *S. apiana, S.canariensis, S. lanigera* and *S. officinalis,* is their antioxidant ability (Al-Hazimi, 1986; Dentali and Hoffman, 1990). Antioxidant components of these species are carnosol, methyl carnosate and carnosic acid (salvin), the foremost of which is reported to be more potent than the synthetic antioxidant, butylated hydroxytoluene (Djarmati *et al.,* 1992). Recent reports identified the monoterpenoid carvacrol as the antioxidant (and antifungal) agent present in *S. lavandulaefolia* (ssp. oxyodon) and *S. fruticosa* (Adam *et al.,* 1998; Deighton *et al.,* 1993; Svoboda and Deans, 1992; Dorman *et al.,* 1995).

The free radical scavenging effects of *S. miltiorrhiza* root (Danshen), widely used in China in the treatment of heart disease (and containing CNS active constituents), have been studied to assess therapeutic potential, and several compounds identified with significant antioxidant activity. Several quinones isolated from *S. miltiorrhiza* root have demonstrated an antioxidant effect in lard, with dihydrotanshinone I, tanshinone I, methylene tanshinquinone and cryptotanshinone providing significant antioxidant activity. Tanshinone II_A has shown no antioxidant activity (Zhang *et al.,* 1990). Other components of *S. miltiorrhiza* root have displayed antioxidant effects, including salvianolic acid A (a compound found to protect against memory impairment induced by cerebral ischaemia reperfusion in mice (Du *et al.,* 1997)), salvianolic acid B which has radical scavenger abilities (Kang *et al.,* 1997), rosmadiol, rosmanol, rosmariquinone (also known as miltirone), epirosmanol and several other phenolic compounds which are also components of other *Salvia* species and other Labiatae species including *Rosmarinus officinalis* (Culvelier *et al.,* 1994, Kang *et al.,* 1997; Liu *et al.,* 1992; Weng *et al.,* 1992).

Salvia compositus is a herbal mixture of the Chinese herbs *S. miltiorrhiza* and *Delbergia odorifera,* traditionally used for management of coronary heart disease

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(Fan *et al.,* 1979). Investigations suggest this herbal remedy has a potential role in anti-oxidation of lipids (Zhang *et al.,* 1994), and in amelioration of cerebral oedema (Kuang *et al.,* 1995), providing further evidence for therapeutic advantage in cerebral disorders.

Anti-inflammatory Activity

Inflammation is associated with detrimental effects in a wide range of disorders including those of the CNS. There is increasing evidence for a role of immune and chronic inflammatory mechanisms in the neurodegeneration associated with ischaemia and AD—arthritis suffers who take non-steroidal anti-inflammatory drugs (for example aspirin) are reported to have a decreased risk of developing AD (Aisen, 1996; Rogers, 1995; Vries *et al.,* 1997).

There have been many studies regarding the anti-inflammatory activity of plants in general and several constituents, mainly phenolics such as flavonoids, are responsible of the activity. Many *Salvia* species and their constituents are reputed to have anti-inflammatory action, for example *S. lavandulaefolia* (Bartram, 1995; Duke, 1985) and *S. plebeia* which is used in Taiwan as a herbal remedy for inflammation (Hernandez-Perez *et al.,* 1995). The diterpenoid aethiopinone from *S. aethiopis* has central and peripheral analgesic properties (Simic *et al.,* 1997) and tanshinones isolated from *S. miltiorrhiza* root have demonstrated anti-inflammatory activity in mice (Chang *et al.,* 1986). All of these *Salvia* species can be considered useful in the treatment of CNS inflammatory disorders.

Other anti-inflammatory constituents of *Salvia* species include the flavonoids carvacrol, cirsimaritin, eugenol, genkwanin, luteolin, quercetin and salvigenin, the terpenoids thymol, *a*- and *ß*-pinene and rosmarinic acid (Bartram, 1995; Bingöl and Sener, 1995; Cañigueral *et al.,* 1989; Cuvelier, 1996; Kuhnt *et al.,* 1995; Tyler, 1993; Wagner *et al.,* 1986).

Oestrogenic Activity

Oestrogenic agents have recently been suggested to play a role in preventing or delaying neurodegeneration in AD. Women on hormone replacement therapy (HRT) are reported to have a decreased risk of developing AD. Over the last decade there has been increasing epidemiological and pharmacological evidence to suggest that oestrogen interacts with receptors, transmitters and neuronal growth in a fashion that may protect against neuronal loss and even plaque formation (one of the main pathological markers of AD) (Birge, 1997; Mortel and Meyer, 1995; Tang *et al.,* 1996). For example, cholinergic neurons in the brain have oestrogen receptors and if given to rats, oestrogen increases the brain enzyme (choline acetyltransferase) involved in the synthesis of the neurotransmitter acetylcholine.

There are reports of the Oestrogenic activity of *Salvia* (*S. officinalis, S. sclarea* and *S. lavandulaefolia*) and some species are components of preparations used to treat gynecological disorders particularly in Spain (Bartram, 1995; Duke, 1985; Planchon and Bretin, 1946; Reynolds, 1996). *S. fruticosa* may have been the origin of such

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reports as "sage inducing fertility in women" (Rivera *et al.,* 1994). Tanshinones isolated from *S. miltiorrhiza* root have demonstrated weak oestrogenic activity (Chang *et al.,* 1986), that suggests antioxidant and cognition enhancing effects (Birge, 1997; Ruiz-Larrea *et al.,* 1994).

It is interesting that there may be a link between the antioxidant, anti-inflammatory and oestrogenic activity of plant constituents, the majority of which are phenolic in structure and they may act in a synergistic fashion. The monoterpenoid carvacrol which is antioxidant and anti-inflammatory (Adam *et al.,* 1998; Cuvelier, 1996; Deighton *et al.,* 1993; Svoboda and Deans, 1992; Dorman *et al.,* 1995) and oestrogens, that are also phenolic in nature, also have antioxidant action (Behl *et al.,* 1997) These actions are considered of relevance in the treatment of CNS disorders (for example AD).

Action in Cerebral Ischaemia

Disorders of the brain arise if cerebral blood flow is impaired, for example strokes are due to occlusion of the arteries leading to loss of oxygen and brain tissue damage (ischaemia). *S. miltiorrhiza* has been employed for the treatment of cerebral vascular disease, and there are several studies to investigate possible mechanisms for the protective effect of *S. miltiorrhiza* against cerebral ischaemia. *S. miltiorrhiza* root has been implicated in attenuating dysfunction of vasoactive intestinal peptide (VIP) (Kuang *et al.,* 1989), a neuropeptide transmitter distributed within the gastro intestinal tract and CNS. VIP may participate in the changes that occur in cerebral ischaemia. Distribution abnormalities of the neuropeptide transmitter substance P has been associated with some CNS disorders, including AD, and decreased levels of substance P has been suggested as a consequence of substance P neuron damage following cerebral ischaemia (Kuang *et al.,* 1991). *S. miltiorrhiza* root has been implicated in protecting substance P neurones from ischaemia (Kuang *et al.,* 1991), and so may actively protect against cerebral ischaemia and perhaps other CNS disorders via this mechanism.

Other beneficial effects of *S. miltiorrhiza* root against cerebral ischaemia have also been explored. *S. miltiorrhiza* root may inhibit neuronal cell death by inhibition of presynaptic glutamate release (Kuang *et al.,* 1994), a high level of which is associated with excitotoxicity. The neuromodulatory effects of nitric oxide (NO) have been suggested to involve excitatory amino acids which effect brain development, learning and memory and it has been suggested that inhibition of NO formation may explain CNS protective effects observed with *S. miltiorrhiza* root (Kuang *et al.,* 1996a; Moncada *et al.,* 1991). This physiological role may aid the explanation of the beneficiai effects of *S. miltiorrhiza* root on the CNS.

Further investigations indicate *S. miltiorrhiza* root may modify ischaemic cell changes by modulating somatostatin, a CNS neuropeptide implicated in learning and memory (Kuang *et al.,* 1993). *S. miltiorrhiza* root may offer an additional therapeutic approach to management of stroke and ischaemia. Reperfusion to aid recovery of ischaemia can cause further brain damage. During reperfusion, metabolism of free fatty acids from the breakdown of lipid membranes during ischaemia has been

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proposed to generate oxygen free radicals leading to further brain injury (Traystman *et al.,* 1991). *S. miltiorrhiza* root has been shown to offer protection against this process by reducing lipid peroxidation (see below) (Kuang *et al.,* 1996b).

Antidepressive Activity

Depression is associated with a monoaminergic (serotonin, noradrenaline, and dopamine) transmission deficiency. Many antidepressant drugs work by either inhibiting the enzyme monoamine oxidase which breaks down these transmitters (and thus increases their levels thereby facilitating transmission) or by inhibiting the re-uptake of serotonin. Antidepressive actions are reported in many plants and in Europe St John's Wort *(Hypericum perforatum)* is a widely used antidepressant which both inhibits the action of monoamine oxidase and also serotonin re-uptake and its constituents may act synergistically (Öztiirk *et al.,* 1996). *S. lavandulaefolia* and *S. officinalis* (in addition to other *Salvia* species) were recommended in folk medicine for depression (in addition to longevity, hot flushes, headache and anxiety) (Reynolds, 1996) and it may be of value to explore *Salvia* pharmacologically for antidepressant activities.

Hallucinogenic Actions

In Western medicine emphasis is on disease and treatment, in other cultures maintaining health and enhancing the quality of life is equally important. In many cultures plants are used for social/recreational and stimulating purposes. One *Salvia* species is used to produce hallucinations (Grob, 1994). Salvinorin A (Divinorin), a

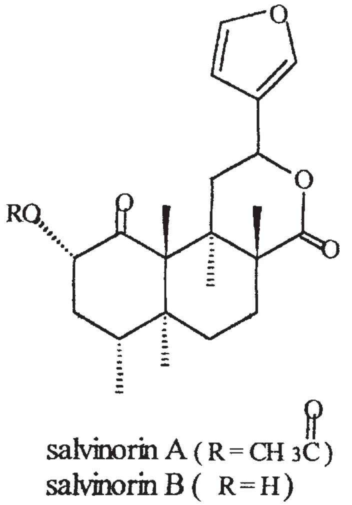

Figure 4 Salvinorum (Divinorum), the diterpenoid hallucinogen present in *Salvia divinorum.*

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(diterpenoid) hallucinogen (Figure 4), is from *Salvia divinorum* and is used by the Mazatec people of Oaxaca in Mexico and in other parts of the U.S. to induce visions (Valdés, 1994). Extracts of wormwood *(Artemisia absinthium)* and other related species (that contain thujone) including *S. officinalis,* have also been used for centuries in Europe to cause hallucinations, though were banned because of their harmful effects on the CNS, such as causing convulsions (Arnold, 1988).

Other Activities that may Enhance the CNS

Inhibitors of rat brain adenylate cyclase have been isolated from *S. miltiorrhiza* root (Kohda *et al.,* 1989). Adenylate cyclase is an enzyme involved in the regulation of various physiological functions, through the production of cyclic-AMP. Cyclic-AMP in turn activates protein kinases (responsible for such functions as enzymes involved in energy metabolism, cell division and ion channel function), leading to changes in neuronal excitability and this may be of relevance in controlling CNS disorders, whereby excitotoxicity can be reduced.

Antimicrobial action (found in *S. fruticosa* and assigned to the monoterpenoid constituents cineole and thujone and the sesquiterpenoid caryophyllene oxide (Moujir *et al.,* 1993; Sivropoulou *et al.,* 1997)), antifungal action (found in *S. fruticosa* and attributed to carvacrol (Adam *et al.,* 1998)), antibacterial action (a common property of terpenoids from *Salvia* (Kuhnt *et al.,* 1995)), antiviral activity (attributed to cineole, thujone, camphor, safficinolide and sageone in *S. fruticosa* and *S. officinalis* (Sivropoulou *et al.,* 1997)) and anti-mutagenic action (attributed to luteolin present *S. officinalis* (Samejima *et al.,* 1995)) may all contribute to the therapeutic effects of *Salvia* species on the CNS whereby the general well-being of the individual is upheld and may even contribute to anti-aging effects.

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VI. BIOTECHNOLOGY
15. THE BIOTECHNOLOGY OF LABIATAE

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INTRODUCTION

Various plant biotechnologies are relevant to the *in vitro* conservation and use of plant genetic resources. These techniques include genetic transformation for the production of modified plants, somatic hybridization using protoplast fusion, *in vitro* methods for fertilization and embryo development, the production of haploid plants *in vitro,* and the production of synthetic seeds using somatic embryos (Lindsey and Jones, 1989). Most of these methodologies are more related directly to the use of plant genetic resources rather than to conservation, although these are potential for the storage of artificial seeds, or the distribution of germplasm in the form of encapsulated embryos or apices (Hasan and Tagaki, 1995). All of these techniques require the use of tissue culture methods, and therefore may be linked with the *in vitro* conservation activities at locations where tissue culture facilities are available.

Genetic transformation protocols allow the introduction of one or a few desirable genes into plant tissues of elite genotypes (Hinchee *et al.,* 1994). This allows for the introduction of new genes without the need for sexual crossing, followed by several backcrosses, which are required in traditional plant breeding methods. Transformed plants have, for example, been produced with introduced insect resistance genes (e.g. *Bacillus* toxin genes), herbicide tolerance and virus protection throught the introduction of virus coat protein genes. A bootlick in the potential generation of genetically modified plants is the availability of genes for desirable agronomic traits. Such genes may be available in germplasm stored *ex situ,* and characterization and location of these genes in stored accessions becomes an important task for the enhanced use of plant genetic resources.

Somatic hybridization methods utilize protoplast fusion followed by regeneration for the production of hybrid plants. These methods also bypass the sexual reproduction process and allow crosses to be made between sexually incompatible plans. The production of asymmetric hybrids through protoplast fusion should allow the introduction of multigenic traits into crop varieties.

In vitro methods for fertilization and embryo development include a range of techniques for assisted sexual reproduction in plants (Raghavan, 1994). These can assist traditional plant breeding involving incompatible hybridizations, where pollen germination, fertilization or embryo development processes may be disrupted. Embryo

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culture, perhaps the most important of these techniques, is used for the rescue of immature embryos or embryos which are arrested in development, following interspecific or intergeneric crosses. In this method, embryos are dissected from the seed and cultured on a solid nutrient medium, which may include endosperm tissue from a normal seed. There are numerous examples of the application of embryo rescue systems for a wide range of important agronomic species. These include *Phaseolus spp.* (Belivanis and Dore, 1986), *Solanum spp.* (Iwanaga *et al.,* 1991) and *Brassica spp.* (Agnihotri *et al.,* 1990). It has been also shown in cassava that embryo culture can yield a higher rate of germination of seeds than standard germination methods (Biigs *et al.,* 1986).

Haploid plants are utilized in plant breeding programs for the production of isogenic lines, and these may be obtained throught the use of anther culture (Dunwell, 1986). Immature anthers containing pollen, inoculated into sterile nutrient medium, can give rise to haploid plantlets which are then used in the generation of isogenic lines. The composition of the medium varies for different species and even for genotypes within species, and has generally been optimized by trial and error. Whereas many important crop species—including rise, wheat, potato and barley—are very responsive to anther culture, many species remain recalcitrant to these methods, and further research is required for these plants.

Synthetic or artificial seeds are produced by the encapsulation of somatic embryos, usually in alginate beads (Redenbaugh *et al.,* 1986). The advantages of these seeds over sexually produced seeds are that (1) they are rapidly produced at any time of the year, (2) they can be produced in large numbers, and (3) they are genetically identical to the original plant. Thus, the major interest is in the commercial production of seeds by a controlled process, with the possibility of production whenever demand occurs. There is considerable potential for the use of synthetic seeds for germplasm exchange, and perhaps for storage of plant germplasm (Seneratha and McKercie, 1989). The latter will require the development of synthetic seeds which can withstand both desiccation and low-temperature regimes.

The biotechnological approach to plant breeding is extremely radical: it operates at a parasexual level. It handles directly the cell and/or the genetic material (DNA) unlike the Mendelian approach working at the whole plant level. Biotechnology has heralded the plant breeding in preparation for the 21st century. As such, the “Gene Revolution” seems to be a possibility in near future.

In this chapter we will try to access the biotechnology research that has been done in various species of the Labiatae family. Before we start referring to specific species we must say that, from a biotechnological point of view, the Labiatae family has not been widely investigated, and that’s why only a small number of related papers could be found. Some data have been reviewed in consideration of the research conducted at the Laboratory of Plant Physiology of the Agricultural University of Athens. The biotechnology of sage is not included in the present review, since this issue is covered extensively by other authors in this volume.

Mentha sp.

Peppermint is a specialty crop of considerable economic value because its oil is used as an additive in cosmetics and organoleptic confectionery (Green, 1985). The

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primary constraint to oil production is crop yield attributable to pests and pathogens. The impact of these biotic stresses is exacerbated by perennial monoculture. Host plant resistance to pests has not been achieved through genetic introgression, since this crop is not very amenable to plant breeding due to sterility and high heterozygosity. New varieties are usually obtained by clonal selection that is facilitated by irradiation mutagenesis of rhizomes (Costabel, 1990). Technological advances in genetic engineering have resulted in the potential for utilizing biotechnology in peppermint crop improvement. The first transgenic peppermint plants have been obtained by *Agrobacterium*-mediated transformation by cocultivation with morphogenically responsive leaf explants. Initial experiments determined that leaves from a 5 cm shoot segnent proximal to the base where most morphogenetically responsive for adventitious shoot formation. Further, explants from the basal portion of the leaf contained cells with greater organogenetic competence than those in the leaf tip. Organogenesis occurred either directly from cells in the explant or from those in primary callus. Temporally, shoot or primary callus formation occurred rather uniformly from regions of the leaf that had been injured as a consequence of dissection during explant preparation (Niu *et al.,* 1998).

Peppermint is one of the plants that synthesize and accumulate various secondary products. The formation of secondary products is regulated in a coordinated fashion, and often in specialized cells or tissues during development. In the case of *Mentha,* specific glands are formed on the surface of the leaves which isolate the toxic monoterpeniod products from the metabolic machinery of the plant. Disorganized plant cell cultures have often proved unsuccessful in the formation of secondary metabolites, particularly complex mixtures such as essential oils. Shoot cultures, derived by transformation with two *Mentha* species, *M. citrata* and *M. piperita,* with *Agrobacterium* based vectors, grow actively as axenic cultures in simple medium, lacking plant growth substances, and produce a range of monoterpenes which reflect the qualitative composition of essential oils from the respective parent plants (Spenser *et al.,* 1993).

Thymus sp.

Thymus is a plant well valued for its essential oil and its uses as a culinary herb. An increasing demand from the flavor industry makes necessary the development of an *in vitro* system for propagation of elite clones. Previous attempts to characterize the *in vitro* behavior of the genus has been restricted to *Thymus vulgaris,* which is a species quite distinct from *Thymus piperella,* a broad-leaved, glabrous, and more herbaceous species (Furmanowa and Oslowska, 1992). According to Saez (1997) the shoot promoting ability was maximum with the presence of BA in the medium. Similarly IAA showed the greatest shoot promoting ability. Best root development was achieved with the presence only of IAA.

Other experiments with thymus, relevant to the analysis of mitochondrial DNA polymorphism in a natural population of *Thymus vulgaris,* revealed the existence of high variability between and among populations. Using southern hybridization with two restricted enzyme length polymorphism analysis and one heterologous probe, 13

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mitnotypes were detected among three natural populations. All the minotypes except two were specific to a single population. This high polymorphism was also deteced with three other heterologous probes. The isolation of restrictable organelle DNA from thyme required severe modifications to the experimental procedure. The cpDNA isolated from three individuals belonging to different natural populations gave identical EcoRI patterns. Chloroplastic membranes seemed very fragile and the DNAse step could not be achieved, even at low concentrations. Belhassen *et al.* (1993) showed that there is a significant variability in the mitochondrial DNA of *Thymus vulgaris* within and among natural populations. In such a case, it is clear that it would be very misleading to name one individual in order to characterize all the species.

Lavandula sp.

Lavandula stoechas is a xerophytic aromatic shrub that commonly occurs in Mediterranean regions, growing in dry conditions and in nutrient-poor and degraded soils. *Lavandula* spp. are some of the most popular medicinal herbs with a great economic interest. The growing demand for natural products has intensified studies towards the selection of native plants and their economic exploitation. *Lavandula spp*. can be vegetatively propagated by stem cuttings or by seeds, but suffer from poor rooting ability of cuttings and growth variation of plants from seeds. These disadvantages can be overcome by using *in vitro* propagation methods. There were no successful reports on *in vitro* vegetative propagation of *Lavandula stoechas* until Nobre (1996) established a method for micropropagation. The progress obtained in the micropropagation of *Lavandula stoechas* suggests the requirement of species-depended conditions for the vegetative propagation of *Lavandula spp*. Bud and shoot induction from callus cultures of *L. latifolia* demanded totally different conditions than in *Lavandula stoechas*.

In vitro cultured cells of *Lavandula vera,* which were being examined as a potential source of biotin (Watanabe *et al.,* 1982) were found to synthesize and excrete a blue pigment into the cultural medium (Watanabe *et al.,* 1985). This was identified by Banhrope *et al.* (1985) as an enol ester of caffeic acid. They also obtained cell cultures from *Lavandula vera* plants that produced phenolic compounds with antimicrobial and antioxidant activities. A methanolic extract from fresh cells of *Lavandula vera* grown in LS medium was used. According to their report the main phenolic compound synthesized by *Lavandula vera* callus culture was mainly rosmarinic acid that was isolated from ethylacetate extracts as a yellowish powder.

Ocimum basilicum

Ocimum basilicum, a popular Labiatae plant known as sweet basil, is used as a kitchen herb and as an ornamental in house gardens. It is known to contain the antioxidant phenolic compound rosmarinic acid, one of the most common caffeic acid esters occurring in the Labiatae family. According to Tada *et al.* (1996) five clones of hairy root cultures of this plant, induced by two strains of *Agrobacteria rhizogenes* and cultured in free-hormone medium, were able to polymerize caffeic acid to a trimer

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(lithospermic acid) and a tetramer (lithospermic acid B), and also produced a high amount of Rosmarinic acid. The results also indicated the importance of the selection of the hairy root clones and culture conditions for the production and the biosynthetic study of successful secondary metabolites in *Ocimum basilicum*.

Hyssopus officinalis

The perennial Labiatae herb *Hyssopus officinalis,* originating from the region of South Europe and Asia Minor, is an important medicinal plant used as an antispasmodic, stomachic, antifungal and for cough treatment. Although the essential oils isolated from these species are popularly available as food and drink additives as well as cosmetic materials, the bioactive phenol constituents of this plant have not been investigated in detail. Murakami *et al.* (1998) showed that transformed root cultures of *Hyssopus officinalis* produce high levels of rosmarinic acid and in addi tion related compounds such as lithospermic acid and lithospermic acid B. That indicates that *Hyssopus officinalis* seems to be one of the most suitable materials for the production of these phenolic metabolites.

Coleus sp.

Coleus forskohlii grows wild in the subtropical Himalayas. The plant is valued for the production of the forskolin drug used for the treatment of glaucoma, congestive cardiomyopathy and asthma. The pharmaceutical industry is largely dependent upon wild populations for supply of plant material for forskolin extraction. Due to large scale and indiscriminate collection of wild material from forests, and insufficient attempts either to allow its replenishment or its cultivation, *C. forskohlii* is rapidly disappearing. Thus, it is considered urgent to develop methods for the conservation of this species. *In vitro* micropropagation techniques offer powerful tools for plant germplasm conservation. Explants from mature potted plants were cultured on MS medium with various growth regulator treatments. Multiple shoot production and rooting were achieved with the presence of auxins (IAA and IBA) in the medium.

Cell cultures of *Coleus blumei* are known to produce high amounts of rosmarinic acid. For investigations concerning localization, transport and accumulation of Rosmarinic acid, Hausler *et al.* (1993) established an efficient method to isolate protoplasts and vacuoles from cell suspension cultures. The isolation of protoplasts was optimized with respect to high yields of protoplasts with good purity in a short time. For the isolation of vacuoles approximately 10^7 protoplasts were subjected to lysis by osmotic shock in combination with EDTA and a basic pH. The rosmarinic acid content of the vacuole was 2.3 times than in the protoplasts. Considering these facts it has been inferred that the rosmarinic acid is localized exclusively in the vacuoles.

Origanum vulgare

In vitro culture of plant cells and organs on chemically defined media offers the opportunity to regenerate and select plants with desirable characters which are

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otherwise difficult to obtain by traditional plant breeding. It is not an easy task to propose a geralized medium for achieving totipotency in all plants or even in all cells of the same plant, as the kind and balance of growth regulators needed for various morphogenic phenomena vary from tissue to tissue and cell to cell depending to their metabolic status. *Origanum vulgare* is an underexploited medicinal plant, a source of the oil of origanum. It is used in high grade flavour preparation, perfumery, cosmetics and liquer industries. Kummari and Saradhi (1992) manage to standardize a media for rapid multiplication of this plant through regeneration of plant from callus. According to their research, the percentages of cultures showing callus induction and root initiation increased with an increase of NAA concentration in the medium. Calli obtained in media supplemented with 2,4-D varied from compact, nodular, friable, to gelatinous depending on the concentration of 2,4-D. For example at higher concentrations of 2,4-D calli were friable and gelatinous. When calli were transferred onto media with BAP, alone or in combination with NAA, formation of localized green patches occured on the calli which formed shoots within a few days. Rooting of the shoots was achieved in B_5 liquid medium supplement with either IBA or NAA, two auxins which are widely employed to induce rooting.

CONCLUSION

Although only a small number of investigators, all over the world, carry out experiments with Labiatae species, the importance of this aromatic plant family is widely known and the antioxidant activity of the Labiatae species can be applied in food, cosmetic and pharmaceutical industry. Lately synthetic antioxidants have provided a point of criticism, in relation to the possible effects of their use on public health. The most common and effective synthetic antioxidants, such as BHT and BHA, have been furthermore blamed to exert toxic activity (Osawa *et al.,* 1992). Therefore, and in the widespread belief that natural products are anyway healthier and safer than synthetic ones, the importance of finding out vegetable compounds able to take effectively the role of conventional antioxidants, and their investigation and economical verification, is widely increasing. Both the food and the cosmetic industry are therefore deeply interested in finding out antioxidant products which, besides retaining pleasant organoleptic characters, do not generate irritative phenomena.

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16. *IN VITRO* ROSMARINIC ACID PRODUCTION

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INTRODUCTION

Higher plants are able to produce particular chemical compounds that are sometimes specific to a species or several species and referred to as secondary metabolites. A plant can produce them in different organs or synthesis may be limited to certain organs or to certain cells of an organ. It is considered that in most cases a certain degree of morphological and/or biochemical differentiation is required for the production of secondary metabolites. The production of these substances in plantlets, isolated organs or cell cultures can be observed under *in vitro* culture conditions.

Among these substances, sage produces the components of essential oil and terpene compounds that have been the subject of much work (Chapters 2, 4 and 5). Another compound of this type—rosmarinic acid—is also synthesised.

Rosmarinic acid is a phenylpropanoid whose chemical structure was identified in rosemary extracts (Scarpati and Oriente, 1958). The metabolite has also been found in other species of Lamiaceae and certain Boraginaceae and Apiaceae (Lamaison *et al.*,1990).

The interest shown in this metabolite is based on its antioxidant properties. Rosemary extract possesses a good antioxidant capability for fats and isolated rosmarinic acid was found to be one of the compounds that give it this property (Hartmann, 1981, Cuvelier *et al.*, 1994, Frankel *et al.*, 1996).

Elucidation of the biosynthesis pathways of this metabolite was undertaken on mint plants (Ellis and Towers, 1970). The subsequent biochemical studies were performed on callus or cell cultures; this material is easier to use than whole plants for experimental purposes. Cell suspensions of certain species can produce rosmarinic acid. Production trials have been undertaken on cell suspensions of *Coleus blumei* (Ulbrich *et al.*, 1985) with a view to industrial use.

BIOSYNTHESIS OF ROSMARINIC ACID AND DEMONSTRATION

Biosynthesis of rosmarinic acid involves the phenylpropanoid pathway. Ellis and Towers (1970) proposed a confirmed biosynthesis pathway in 1970 using *Mentha* plants. This was confirmed several years later by Razzaque and Ellis (1977) with cell suspensions of *Coleus blumei*.

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Two precursors—tyrosine and phenylalanine—are involved in this biosynthesis procedure. Dihydroxyphenylalanine (DOPA) was used as the first intermediary after tyrosine in the first biosynthesis chain. Subsequent studies made it possible to refine the first method proposed and, in 1979, ELLIS *et al.* proposed a chain in which 4-hydroxyphenylpyruvic acid is the intermediary following tyrosine. The enzymes involved were shown and isolated subsequently. In 1989, the last enzyme in the chain, rosmarinic acid synthase, was isolated and described by Petersen. The mechan isms regulating rosmarinic acid synthesis are still unknown. The chain requires reduction capability in the form of NADPH and NADH. It seems to function in such a way as to keep the release of intermediate substances to a minimum and there is good connection between free precursor amino acids and the rosmarinic acid synthesis chain (De-Eknamkul and Ellis, 1989). This research essentially concerns cell suspensions of *Anchusa officinalis* and *Coleus blumei* (see Petersen *et al.*, 1994 for a review).

PRODUCTION USING *IN VITRO* CULTURES OF SAGE

Initiation of Cell Cultures

Callus was initiated using *Salvia officinalis* leaves removed from shoot tip culture plantlets that had not yet rooted. The mineral media used are those of Murashige ans Skoog (1962) (MS) and B5 of Gamborg *et al.* (1968). To these were added the vitamins described by Nitsch *et al.* (1968), 88 mM sucrose, 0.8% gelose (W/V) and plant growth regulators: 2,4-D and kinetin, a-naphtalenacetic acid and benzyl adenine (BA) or pichloram from 0.25 to 22 μM. The pH was adjusted to 5.3 before autoclaving. Calli were transferred after culture for 4 to 5 weeks to 250 ml or 500 ml conical flasks containing an identical medium without gelose. Subcultures were performed every 14 days for the cultures on MS media and every 10 days on B5 media, with 30 or 60 ml suspension placed in 80 or 160 ml fresh medium. The flasks were kept on a rotary agitator (20 rpm^{-1}) in continuous light (24 to 38 $\mu M.n^{-2}s^{-1}$) at 23 °C. The suspensions cultured on MS media were chloro phyllous and photomixotrophic and those on B5 were non-chlorophyllous. The results for the three following culture media are described:

MSA: MS—2.26 μM 2,4-D—2.32 μM kinetin,
MSB: MS—2.07 μM pichloram,
B5A: B5—2.26 μM 2,4D—2.32 μM kinetin.

As rosmarinic acid is not excreted into the culture medium, suspension aliquots were removed at different stages of culture. The cells were filtered under vacuum, cooled with liquid nitrogen and then freeze-dried. The lyophilisate was extracted three times for 20 min with 70% ethanol. The extract was evaporated to dryness and resuspended in methanol. The samples were analysed by HPLC on a C18 DB column (Supelco, U.S.A.) at 328 nm. An acetic acid-methanol-water mixture was used as eluent. Acetic acid was used at 5% and the methanol:water gradient varied from 85:10% to 0:95%. Analysis time was 25 min.

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The extract can also be separated by thin layer chromatography, with detection being by spectrophotometry at 328 nm.

Growth and Production under Standard Culture Conditions

Growth on callus varied according to culture medium and hormonal balances. Rosmarinic acid production also varied from 0.005% fresh weight on an MSA medium to 0.06% fresh weight on B5A medium. On MS medium, production from callus was much more stable in time than on B5 media where production varied by as much as 50% (Hippolyte, 1990).

Under maintenance conditions (Fig. 1), the growth, rosmarinic acid production and interactions between growth and production of cell suspensions also differed in the various media.

Maximum growth and production were attained between day 10 and day 15 on MSB medium and on day 15 and day 20 on MSA and B5A medium, respectively. Production on MSA and MSB starts from the beginning of the culture. On B5A, there is a latency period of at least 5 days. Rosmarinic acid (RA) production is twice as great on medium B5, but this is related to strong growth on this medium with equivalent RA production related to dry weight on the three culture media (approxi mately 5%) (Hippolyte *et al.,* 1991). Work by Whitaker *et al.* (1984) on *Salvia officmalis* and *S. triloba* cell suspensions showed that rosmarinic acid production varied from 0.9 to 7.8% dry weight on the same culture medium, depending on the varieties used to initiate the cell suspensions. These differences can also be seen when two species are grown on the same medium. In callus cultures of *S. officmalis* and *S. fruticosa,* Kintzios *et al.* (1996, 1998) observed that peak production and growth could double from one species to another. The production pattern was also different in the two species.

Stimulated Production Conditions

The MSA medium was used for these tests; the medium had been studied in a 2 L fermenter (Biolafitte), where the suspension displays the same growth and production kinetics as in a conical flask. Production tests were performed on one-year-old cell suspensions. The behaviour of the suspension was studied in a culture cycle in a medium in which some components had been modified. Modifications consisted of adjustment of the sucrose content (88 to 146 mM), the addition of phenylalanine at different concentrations (0.1 to 0.75 g/l) to standard or sucrose-enriched medium and transfer to a low medium proposed by Heller (1953) with low osmolarity and enriched with sucrose to 146 mM. The effect of the medium on the crop cycle was studied in all cases.

Effects of modifications to the media on growth and production peaks

The addition of phenylalanine to a control culture medium enhances production and growth (Hippolyte, 1992). This effect is directly correlated with the concentration of the substance and the most marked effect is observed at 0.75 g/l, the highest

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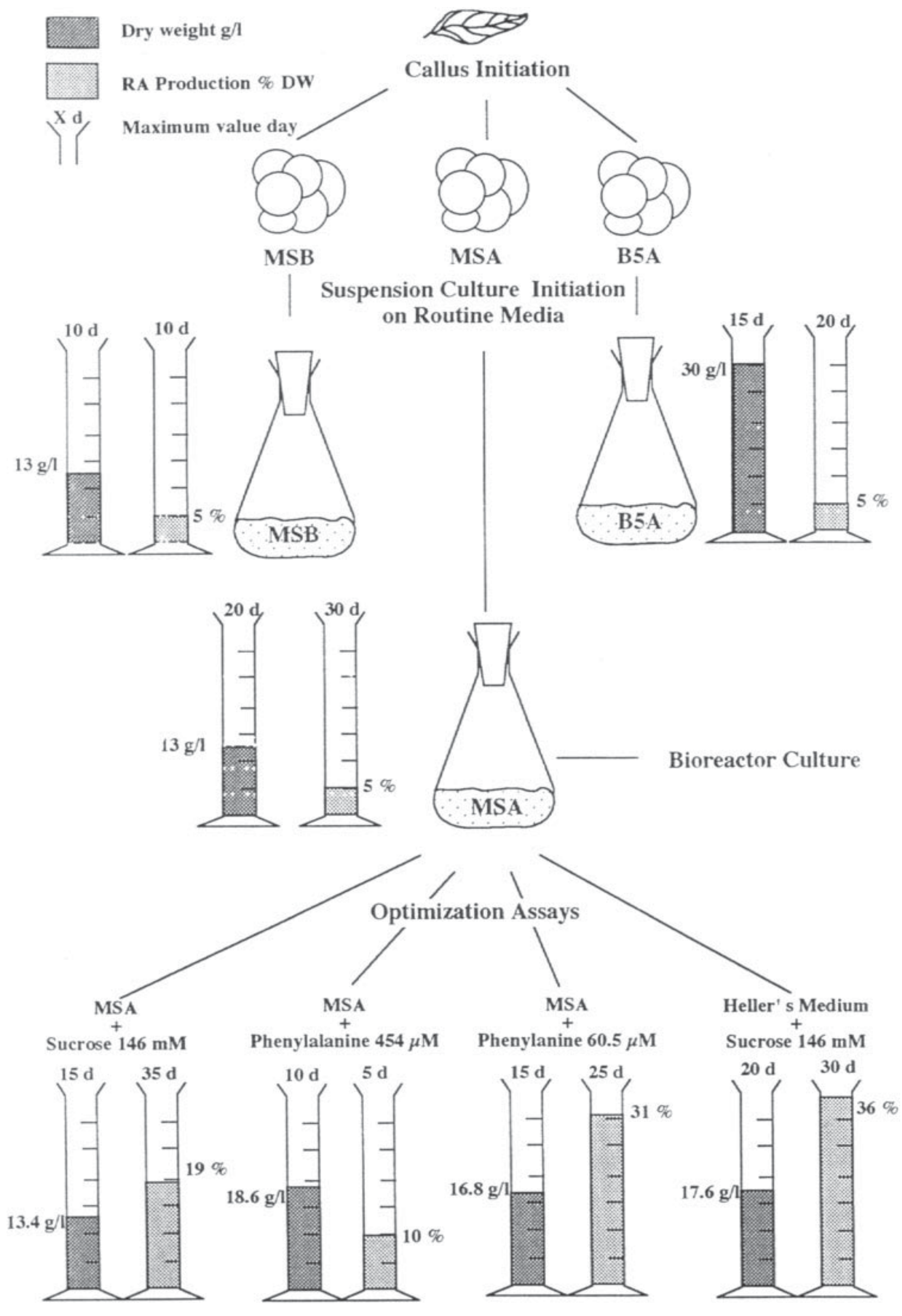

Figure 1 Major antioxidative compounds in *Salvia officmalis*.

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concentration (Fig. 1). The enrichment of all MS media with sucrose had little effect on growth of the suspension in comparison with the control. A slight increase in dry weight in comparison with the control was observed on Heller's medium.

In contrast, production peaks were substantially influenced by the addition of sucrose to MSA medium, with an increase from 4.5% to 19% dry weight. The addition of 0.75 g/l phenylalanine doubled cell productivity in comparison with the control. However, combining 146 mM sucrose and a low phenylalanine concentration (0.1 g/l) resulted in production of 6.4 g per litre, representing 36% of the dry weight MS. The same results were obtained on Heller's medium enriched with sucrose.

Effects of modifications of the medium on growth and production kinetics

Increasing the sucrose concentration has little effect on the time required to attain growth peaks. A difference of 5 days was observed, which may be a result of the sampling frequency. The same was observed on Heller's medium (Fig. 2).

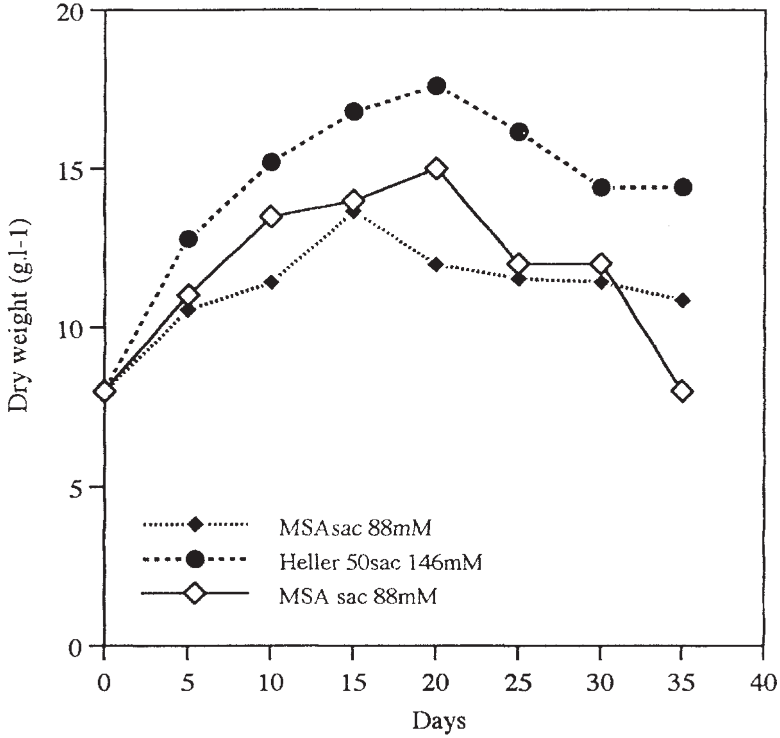

Figure 2 Cell growth (dry weight g.1^{-1}) of *Salvia officinalis* cell cultures on basal MS medium containing 88 mM (♦), 146 mM (•) sucrose and Heller's medium containing 146 mM surose (•)

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In contrast, the addition of phenylalanine to the standard medium strongly reduced the time required to attain peak levels. Indeed, the standard medium containing 0.75 g/l phenylalanine made it possible to attain peak RA synthesis after 5 days and peak growth after 10 days in comparison with 25 and 20 days in the control.

In all the MS media enriched with high osmolarity sucrose (238 mOsmol), with or without phenylalanine, production only started after the halting of growth, whereas production was simultaneous with growth in the control (181 mOsmol) and on Heller's medium (185 mOsmol) (Figs 2 and 3).

In 1977, Zenk showed that the most favourable sucrose concentrations in cell suspension of *Coleus blumei* were between 5 and 7%. In the same species, the increase in the sucrose concentration from 2 to 4% causes an increase in rosmarinic acid production from 2 to 21% of the dry weight (Gertlowski and Petersen, 1993) without modification of the production procedure but with a longer cycle. In this case, the sucrose absorption rate remains constant, whatever the concentrations and types of sugars. In contrast, during the first 10 days of culture of *S. officinalis,* the

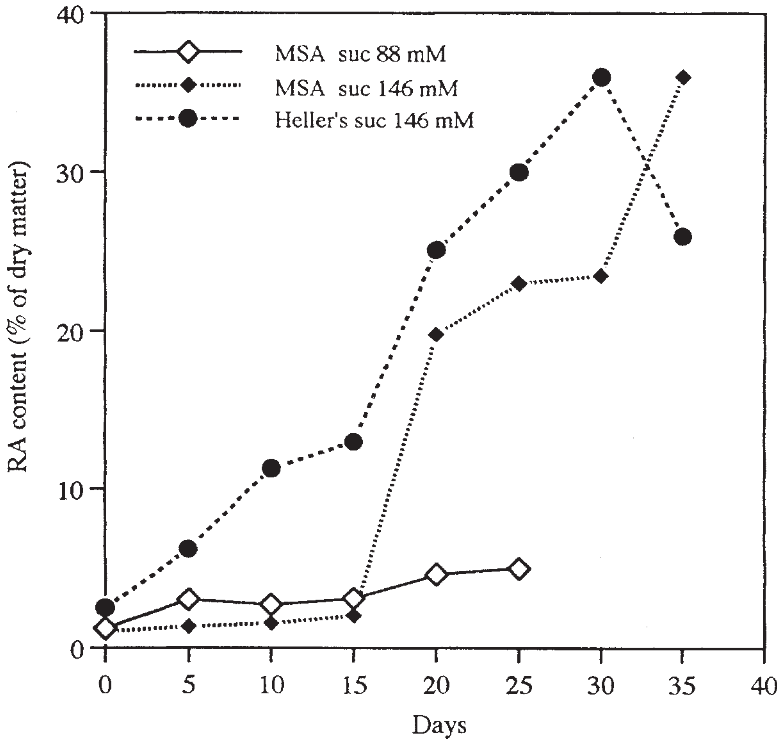

Figure 3 RA production (% dry weight) of *Salvia officinalis cell* cultures on basal MS medium containing 88 mM(•), 146 mM (•) sucrose and Heller's medium containing 146 mM sucrose (•).

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curves for disappearance of sucrose from the culture media containing 88 and 146 mmol of the sugar were similar and then absorption was very marked from day 10 to day 15 of culture—corresponding to the start of synthesis—in the latter medium (Hippolyte, 1990).

With *Anchusa officinalis,* enriching the medium with sucrose has a negative effect on production and the most favourable sucrose concentration is 3% (De-Eknamkul and Ellis, 1985).

Composition of Extracts

Work reported on Lamiaceae mainly concerns undifferentiated cells such as callus and cell suspensions. Cell suspensions of *Salvia officinalis* produce rosmarinic acid in measurable quantities (Hippolyte, 1990). After several subcultures, callus of *Salvia miltiorrhiza* produces only rosmarinic acid whereas callus newly initiated from petioles (little dedifferentiated cells) produces RA and lithospermic acid (Morimoto *et al.,* 1994). Cell extracts of other species may contain other phenolic compounds in addition to RA. Cell cultures of *Lavandula vera* produce small quantities of caffeic acid, one of the intermediate substances in the biosynthetic chain (Kovatcheva *et al.*, 1996), those of *Coleus blumei* synthesise unidentified phenolic compounds but in very small quantities (Razzaque and Ellis, 1977).

At a higher level of morphological differentiation, root cultures of *Ocimum basilicum* induced by *Agrobacterium rhizogenes* produce rosmarinic acid and also lithospermic acid (up to 2% of the dry weight) and lithospermic acid B (0.15% of the dry weight) (Tada *et al.,* 1996).

CONCLUSIONS

The results of RA production by *in vitro* cultures of plants of the genus *Salvia* are shown in Fig. 1. The studies performed on cell suspensions of *Salvia officinalis* display interesting characteristics. Firstly, extremely large *in vitro* production of a metabolite was observed under stimulation conditions. To the best of the author's knowledge, this species gives the largest quantities assayed, with 6.4 g/l representing 36% of the dry weight.

It would seem that the biosynthetic pathway is such as to minimise release as biosynthetic intermediaries were not detected in the extracts. The state of morphological non-differentiation of cells induced by suspension culture seems to enhance essentially the production of this metabolite.

It has also been seen that the mode of production varies from one medium to another. It is difficult to determine the role of phenylalanine as a precursor. Indeed, the increase in the phenylalaline concentration shows that it serves as a limiting factor for production and, to a lesser extent, growth, under standard culture conditions. It also has a substantial effect on growth and production mechanisms by considerably shortening the culture cycle. The limiting effect of a precursor has often been observed in studies of the production of secondary metabolites (Margna, 1977). However, in a sucrose-enriched medium, a synergistic effect is observed on

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production only for a small concentration of this substance; growth and the culture cycle are unchanged (Hippolyte *et al.,* 1992). The effect of the substance seems to be masked by the sucrose effect. The addition of phenylalanine for other species gives contradictory results. Indeed, it reduces RA production by 35% in suspensions of *Coleus blumei* added to a standard medium (Razzaque *et al.,* 1977), but improves it by 100% when it is added to a medium containing insufficient sucrose (Zenk, 1977). This observation corroborates the results of De-Eknamkul and Ellis (1985): combining the most favourable factors does not necessarily give the best results.

Sucrose has a substantial trophic effect and also an osmotic effect. It would seem that both effects were related to the production of rosmarinic acid in our cell suspensions. On the one hand, the trophic factor affects the quantity of rosmarinic acid produced. Very large quantities of RA were assayed; they were similar in MS medium and Heller's medium enriched with sucrose. The effect of sucrose alone on osmotic pressure is difficult to demonstrate. The conditions that we chose in order to maintain an osmotic pressure identical to that of the standard medium while increasing the sucrose content of the medium (i.e. by decreasing the molarity of the mineral medium) showed the effect of osmotic pressure in sucrose-enriched MS medium on production quality rather than quantitity after growth when the osmotic pressure increases. Unfortunately, under these conditions, the possibility that the modifications might be related to the composition of the culture medium cannot be ruled out, even though no phenomena of this type were observed on MS medium and B5 medium, which differ particularly in their nitrogen concentrations.

Coleus blumei cell cultures may also display differences in growth and production kinetics. The suspensions maintained by Gertlowski and Petersen (1993) synthesised RA at the end of the growth phase with low and high sucrose concentrations, whereas Razzaque *et al.* (1977) observed production starting in the linear growth phase. However, when the latter authors added NAA instead of 2,4D to the culture medium, RA production began at the beginning rather than the end of the exponential growth phase. Finally, still on the subject of *Coleus blumei* cell suspensions, the production system developed by Ulbrich *et al.* (1985) induces RA production when growth has been completed.

The hypothesis of Sakuta and Komamine (1987) according to which secondary metabolites may belong to two categories according to their mode of production (i.e. those whose production is growth-related and those whose synthesis takes place after growth and is dependent on cell differentiation) does not seem to apply in this case. Here, the connection between primary metabolism and secondary metabolism seems to be caused by pressure in the culture medium in which osmotic pressure may play a regulatory role. Other factors may also be involved such as the type of hormones. Genetic factors may also determine the mode of production (Kintzios *et al.,* 1996, 1998).

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17. *SALVIA* SPP.: TISSUE CULTURE, SOMATIC EMBRYOGENESIS, MICROPROPAGATION AND BIOTRANSFORMATION

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INTRODUCTION

Although *Salvia,* one of the most commercially important and widely cultivated medicinal plants, is a perennial, it does not last above three or four years without degenerating, so that plantations should be renewed at least every four years (Grieve, 1994). In addition, and in spite of the fact that considerable progress has been made in the field of the *in vitro* production of various secondary metabolites, such as rosmarinic acid and cryptotanshinone (see I. Hippolyte and Em. Panagiotopoulos, in this volume), the application of biotechnological methods for the propagation of these species is rather limited. This might be due to the fact that most *Salvia* species can be easily propagated by cut tings and layers, most frequently in the spring and in the autumn, by pulling off or pegging down shoots from three-year-old plants (Grieve, 1994). Some species, like *S. sclarea,* the clary sage, are propagated by seed, but for most species this method of propagation is limited by the rather low seed germination rate.

There is, however, an increasing interest in the development of efficient protocols for the tissue culture and micropropagation of certain *Salvia* species, in order to establish a relatively fast system for producing disease-free and true-to-type clonal (and therefore uniform) plants from outstanding genotypes. In addition, there is substantial evidence that plant secondary metabolite production can be enchanced by the *in vitro* induction of morphogenesis, so that *in vitro* regenerated plants can be a useful resource for pharmacologically active compounds.

Tissue culture techniques could also be invaluable for the conservation of endangered or rare *Salvia* species, such as *S. pratensis,* a perennial restricted to a few isolated populations in the Netherlands (varying in size from 10 to 1500 flowering plants) (Ouborg and Van Treuren, 1995).

The present report focuses on a concise presentation of the various methods developed for the induction of callus, organogenesis and somatic embryogenesis as well as plant regeneration for micropropagation and breeding purposes of some *Salvia* species. Furthermore, the accumulation of secondary metabolites (in particular rosmarinic and lithospermic acid) in *in vitro* differentiated tissues is reviewed.

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GENERAL

Optimization of the tissue culture procedure in *Salvia* has called for the employment of different culture media (especially growth regulators) and culture conditions (such as light and temperature) for each separate species. However, some features of the culture process are essentially the same for most of the cases studied:

Explant Source

Several kinds of explants have been used for the establishment of tissue cultures from *Salvia* species, such as seeds (*S. miltiorrhiza*—Waldemar, 1996), shoot tips (*S. miltiorrhiza*—Morimoto *et al.,* 1994), shoots with axillary buds (*S. canariensis*—Luis *et al.,* 1992), young leaves (*S. officinalis and S. fruticosa*—intzios *et al.,* 1996, 1998), embryos (interspecific species of *S. sclarea*—Rusina *et al.,* 1997) and seedlings from *in vitro*-germinated seeds (*S. miltiorrhiza*—Miyasaka *et al.,* 1989, Gao *et al,* 1996).

Explant Disinfection

Seeds and shoot explants have been regularly surface-sterilized in 1–2% solutions of sodium or calcium hypochlorite (15 min-1 hr) usually followed by immersion in 70% ethanol (30s). Luis *et al.* (1992) additionally used Benlate (at a concentration of 1g/l) for the surface-sterilization of stem explants from mature *S. canariensis* plants. Finally, a surface-sterilization for 12 min in 0.1% (w/v) mercuric chloride solution, containing 1–2% Tween-80, is recommended for leaf explants (Kintzios *et al.,* 1996, 1998) derived from field-grown plants.

When tissue-culturing certain *Salvia* species, explant browning can be an serious problem: explants develop necrotic areas which in some cases lead to expiant decline and death. This effect is both genotype- and culture medium-dependent: For instance, in *S. officinalis,* application of a-napthaleneacetic acid (NAA) and 6-benzyladenine (BA) generally stimulated callus formation, but also promoted explant necrosis (Kintzios *et al.,* 1996). For the same species, the development of necrotic symptoms on explants was highly negatively correlated with callus induc tion. This effect was more profound at low light intensities (50 μmol m^{-2} s^{-1}). On the contrary, browning of the *S. fruticosa* explants did not have any observable effect on callus induction: almost all declined explants were able to dedifferentiate as a response to culture. The addition of ascorbic acid (at an optimal concentration of 10 mg/l) to the culture medium greatly reduced the frequency of the necrotic symptoms.

Culture Medium

The basal medium of Murashige and Skoog (MS medium) (1962) has been most frequently employed in the tissue culture of *Salvia.* Other types of media (usually of a lower ionic strength), such as Almacigo's medium (Mederos and Lopez, 1991),

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White medium (White, 1943) or B5 medium (Gamborg *et al.,* 1968) have been occasionally used.

Culture Conditions

For culture initiation, explants of various *Salvia* species have been incubated at 23–25 °C, over a 16 hr photoperiod and under a photosynthetic photon flux density (PPFD) of 25–250 µmol m^{-2} s^{-1} (depending on the particular species).

SPECIES-SPECIFIC PROTOCOLS

Salvia miltiorhizza

Callus induction

Callus cultures of *S. miltiorrhiza* have been successfully induced from *in vitro* grown seedlings (epicotyles and hypocotyles) (Miyasaka *et al.,* 1989, Waldemar, 1996) and shoot tips (Morimoto *et al.,* 1994) on a solid MS medium supplemented with either 4.5 µM 2,4-dichlorophenoxyacetic acid (2,4-D) and 0.5 µM kinetin (Kin) (seedling tissues) or 2.5 µM indolebutyric acid (IBA) and 1.3 µM BA (shoot tips) (usually under a PPFD of ca. 170 µmol m^{-2} s^{-1}). In the latter case, adventitious shoot induction was always concomitant with callus formation, but replacement of IBA with 2,4-D enhanced the further induction of callus from *in vitro* regenerated petioles.

Shoot and bud induction

Shoot structures have been induced either through callus or directly on cultured explants. Regeneration of shoots from callus cultures has been achieved on solid MS medium supplemented with either 0.5 mg/l indoleacetic acid (IAA) and 4.6 µM Kin (seeling-derived callus) (Waldemar, 1996) or 4.6 µM Kin and 1.4 µM gibberellic acid (GA_3) (formation of multiple shoot complexes from petiole-derived callus), under a PPFD of ca. 170 µmol m^{-2} s^{-1} (Morimoto *et al.,* 1994).

Seedling tissues (from sterile germinated *S. miltiorrhiza* seeds) have been used for the direct induction of adventitious bud clumps on MS medium supplemented with 2.6 µM BA and 0.5 mg/l IAA, under a PPFD of 36 µmol m^{-2} s^{-1} (Gao *et al.,* 1996).

Root induction

Rooting of shoot cultures (several weeks-old) has been usually achieved on a growth regulator-free solid MS medium (Morimoto *et al.,* 1994, Waldemar, 1996) or on solid B_5 medium with 1 µM IBA (Gao *et al.,* 1996).

Regenerated plants were potted in either soil-leaf mould (1:4) or vermiculite and cultivated for 3–10 weeks (23–25 °C, 16:8 h, 125–200 µmol m^{-2} s^{-1}) before being carried over to the greenhouse or the field for further hardening and cultivation.

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Tissue culture has been proven a very efficient propagation method for *S. miltiorrhiza*. Fifty four fertile plants could be regenerated from a single seed (Waldemar, 1996) while more than 10^7 clonal plants could be theoretically obtained from a single shoot tip during a total period of 33–37 weeks (including the time necessary for hardening transplanted regenerated plants).

Secondary metabolite accumulation

Aerial parts of both seed- and stem regenerated *S. miltiorrhiza* plants demonstrated a higher content of phenolic acids (rosmarinic and lithospermic acid) than parental plants. The inverse was true for the roots, but, as Morimoto *et al.* (1994) mentioned, this could simply be due to the age difference of the plant material: parent plants were much older (at least three-years old) than regenerants (only a few weeks old). Accumulation of phenolic acids was essentially the same in shoots either regenerated *in vitro* from callus cultures or clonally propagated from shoots (Morimoto *et al.*, 1994). Interestingly, accumulation of phenolic acids in regenerated plants transferred after rooting to vermiculite for 5 weeks, then cultured in soil for another 5 weeks, was higher than in plants cultivated continuously in vermiculite only. Callus cultures themselves, on the other hand, demonstrated a rather poor accumulation potential, which was ca. 3–4 times lower than in regenerated plants.

Salvia officinalis and *S. fruticosa*

The common sage is the most representative of the *Salvia* species. It has been cultivated for culinary and medicinal purposes for many centuries in Europe and Middle-East (Grieve, 1994). It is a very variable species, possessing remarkable curative properties. Many kinds of sage have been used as substitutes of tea.

Salvia fruticosa is a sage species endemic to the Mediterranean region, commonly used as a substitute of tea.

Callus induction and somatic embryogeneis

Kintzios *et al.* (1996, 1998) reported on the induction of somatic embryogenesis from leaf explants of *Salvia officinalis* and *S. fruticosa* on a MS medium supplemented with 1.8–18 µZM 2,4-D and Kin or 10.5–21µ M NAA and BA. Only explants from young plants (having 6–8 leaves) responded to the culture treatments and, in general, low light intensities (50 µmol m^{-2} s^{-1}) favoured callus formation and induction of somatic embryos. S. *fruticosa* responded to culture much better than *S. officinalis*. Callus tissue was formed on explants of this species at a 95–100% rate under both low and high light intensities. The callus derived from *S. fruticosa* also grew at a faster rate than the one from *S. officinalis* explants (Fig. 1). Higher callus induction rates were obtained for *S. officinalis* when equimolar auxin and cytokinin concentrations were used. This was also the case for 2,4-D and Kin, but only when applied at the lowest (1.8 µM) or the highest (18µM) concentrations. A novel growth pattern was observed *for S. fruticosa* callus: it maintained a much higher growth rate

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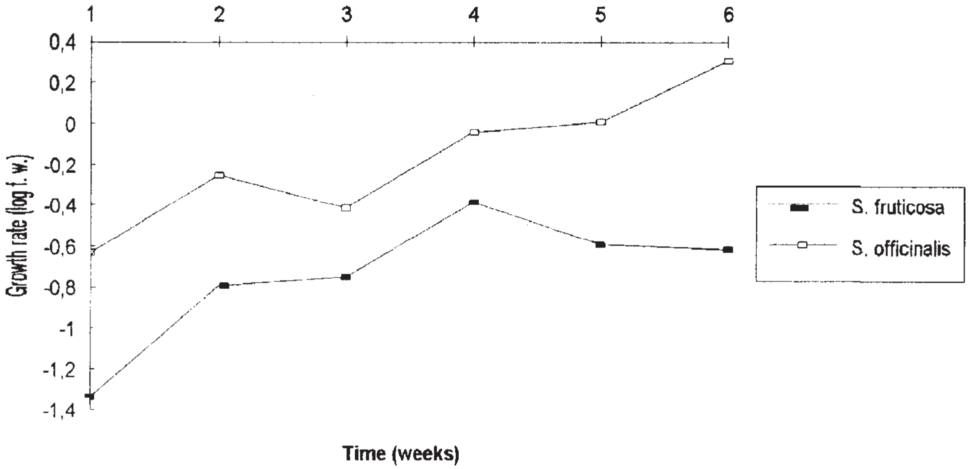

Figure 1 Growth rate of *S. officinalis* and S. *fruticosa* callus cultures on MS medium supplemented with 4.5 µM 2,4-D and 4.5 µM Kin.

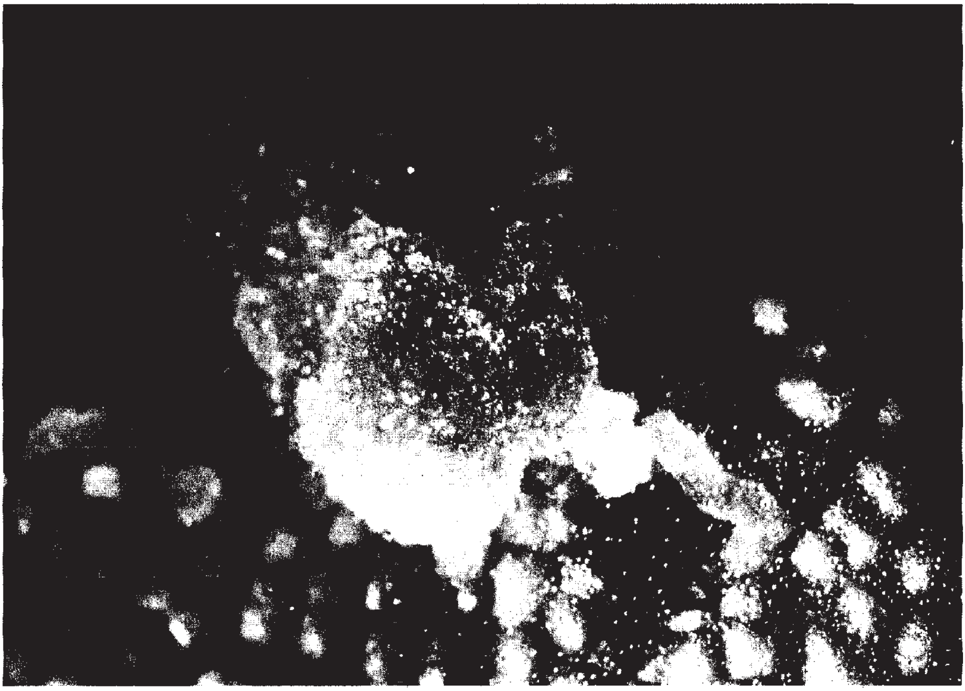

Figure 2 Embryogenic callus of *S. officinalis* 2 weeks after culture initiation on MS + 4.5 µM 2,4-D+4.5 µM Kin (scale bar=0.8 mm).

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throughout the period of investigation with only an intermediary slight growth reduction between the 2nd and the 3rd week after callus formation. For the latter species, higher PPFD values had an observable beneficial effect on callus proliferation.

Globular somatic embryos were readily formed on callus tissue of both species after 3 weeks in culture (Fig. 2). Only *S. officinalis* embrya, induced on 10.5 µM NAA and 10.5 µM BA, and *S. fruticosa* embrya, induced on 10 µM NAA and 21 µM BA, were able to further develop on the same medium until heart- and torpedo-shaped forms (1–2 mm long) appeared. On the contrary, globular embryos of both species induced on 4.5 µM 2, 4-D and 4.5 µM Kin developed further only after being transfered to a medium with no growth regulators at all. The PPFD value had no effect on the maturation process. In every case, 1–2 mature embryos were counted per callus tissue.

Secondary metabolite accumulation

Maximum rosmarinic acid accumulation in *S. officinalis* callus cultured on 4.5 µM 2, 4-D and 4.5 µM Kin coincided with the onset of somatic embryo induction (e.g. at the beginning of the 3rd week after culture initiation), as well as during final embryo development and maturation (6–7 weeks after culture initiation) (Fig. 1). Thus, the process of tissue redifferentiation in vitro was assossiated with an enhanced phenolic acid accumulation, as already aforementioned for *S. miltiorrhiza*.

Salvia sclarea

Salvia sclarea, the clary sage, is a biennial plant yielding an oil (clary oil) with a highly aromatic odor, which is under increasing attention for its use in aromatherapy. After the essential oil is removed the crude material is a source of sclareol which is converted to the sclareolide; both are used commercially in the manufacture of ambergris perfumes and as inhibitors of growth of rust fungi (Grieve, 1994, Dweck in this volume).

Banthorpe *et al.* (1990) reported on the establishment of undifferentiated friable, white callus and derived cell suspension lines from stem explants of a sterile *S. sclarea* plant on a MS medium supplemented with either 4.5 µM 2, 4-D and 0.5 µM Kin or 5.4 µM NAA and 0.5 µM Kin. A callus induction rate of ca. 80% was observed. Consequently, cell suspensions were established on MS liquid medium supplemented with 4.5 µM 2, 4-D and 0.5 µM Kin. The cultures were used in order to study the *in vitro* accumulation of sclareol.

Rusina *et al.* (1997) used the method of isolated embryo culture for producing interspecific hybrids of *S. sclarea* with the wild species *S. scabiosifolia, S. grandiflora* and *S. aethiopis*. Embryo growth *in vitro* and the frequency of hybrid plantlets were affected by the stage of embryo development, the composition of the medium and the genotype of the parents. Optimum for hybrid production were modified White medium and the torpedo stage of embryo development. The percentage of hybrids produced was 12.2 to 38.3% depending on the cross. A study of the plants in the field enabled the selection of those with the highest essential oil content.

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S. canariensis

This species, which is endemic of the Canary islands, contains several diterpenes with considerable antibiotic and antioxidant activities. Luis *et al.* (1992) induced axillary buds on stem segments taken from mature 13-year-old *S. canariensis* plants on a modified Almacigo's medium supplemented with 0.01 µM BA and 0.01 µM NAA. Multiplication and elongation of the axillary buds was achieved on modified MS medium containing lower levels of BA (23–25 °C, 16 hr, 25 µmol m^{-2} s^{-1}).

CONCLUSION

Existing experience with tissue culture of *Salvia* sp. is limited o a small number of species and mainly concerns callus induction from various explants in order to facilitate the *in vitro* production of secondary metabolites. *S. miltiorrhiza* is probably the only species where different approaches to plant regeneration *in vitro* have been successfully taken. However, progress in somatic embryogenesis and recent research on the technology of synthetic seeds, along with other advanced aspects of tissue culture (e.g. protoplast culture and fusion, creation of autotetraploid lines) could offer to a significant involvement of biotechnology to the propagation and breeding of the genus *Salvia*.

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18. PRODUCTION OF SECONDARY METABOLITES USING LIQUID CULTURE OF SALVIA PLANTS: UP-TO-DATE REPORTS AND SCALE-UP POTENTIAL

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INTRODUCTION

Since ancient times, most of the plants in the Lamiaceae family have been mentioned for their pharmaceutical and therapeutical abilities. Usually by mixing with other species or by drinking the extract from boiled plant leafs, they have been used by folk medicine as well as modern medicine until today. As a result, the research concerning *Salvia* plants is focused on the medicinal activity of individual substances that can be found in extracts from aerial parts or from roots of the plant. The majority of the active—worthy of investigation substances are products of metabolic pathways that apparently are not involved directly in growth or development, known as secondary metabolites. Those secondary plant products appear to function mainly as defences against predators and pathogens.

As we can see, there is a great interest in using antioxidant, antimicrobial, antiviral and even anticancer substances that are products of "natural factories", the plant cells. Natural products often have less side effects, when applied in normal dosages and they are more acceptable in a wide range of consumers. The main disadvantage of natural substances is the small quantity that can be extracted from very large quantities of plant parts. Also, some species are difficult to cultivate in a wide range of environmental conditions and if they grow they give a poor product yield. Lining up against those disadvantages, tissue culture and especially liquid culture seem to give a potential to future production of plant secondary metabolites.

SECONDARY METABOLITES OF *SALVIA* PLANTS

Categories and Structure of Secondary Metabolites

There are three major groups of secondary products according to their biosynthetic way: terpenoids, phenolics and nitrogen containing compounds.

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Terpenoids

Terpenoids are lipids synthesised from acetyl-CoA via the mevalonic acid pathway. They consist of five-carbon units that have the structural frame of the isopentane. The taxonomy is based on the number of the C_5 containing units of the terpenoid. So the two C_5-unit containing terpenoids are called monoterpenes (10 carbon atoms), the three C_5-unit containing terpenoids are called sesquiterpenes (15 carbon atoms), the four C_5-unit containing terpenoids are called diterpenes (20 carbon atoms) and so on (triterpens-30 carbon atoms, more than 40 carbon atoms-polyterpenes). Some terpenoids occur in primary metabolism pathways as in-between products of primary production, such as some plant growth regulators (absisic and gibberellic acid) and cell membrane components (steroids from triterpenes). Most of plant terpenes are used as defence molecules having toxic activity that prevent herbivorous insects and higher animals from eating plant tissues.

Phenolics

Phenolic compounds are aromatic substances mainly formed via the sikimic acid or the malonic acid pathway in various ways. This group has the characteristic of a hydroxyl joined to an aromatic ring. It is a rather heterogeneous group because some phenolics are water soluble, others can be solved only in organic solvents and some are insoluble polymers. They play various roles in the plant's physiology, such as defence against pathogens and herbivorous, mechanical enforcement and attraction to pollination insects.

Nitrogen containing compounds

Most of those secondary products are synthesized by common amino acids. In this group we can find many plant defense substances, such as alkaloids and cyanogenic glycosides. Plants from *Salvia* species do not contain any worthy substance from this category of secondary metabolite.

Main Secondary Metabolites in *Salvia* Plants

The following compounds of *Salvia* plants that belong to the phenolics group have been studied:

Rosmarinic acid (RA)

Chemically known as a-O-caffeoyl-3, 4-dihydroxyphenillactic acid, rosmarinic acid belongs to the phenolics group and it is characterised as a phenylpropanoid (Fig. 1). It was identified for the first time in rosemary extracts (Scarpati *et al.,* 1958) and it is known for its antioxidant activity. Rosmarinic acid is biosynthesised through the condensation of caffeic acid and 3, 4-dihydroxyphenyllactic acid. Two precursor amino acids—phenylalanine and tyrosine—are involved in this biosynthetic

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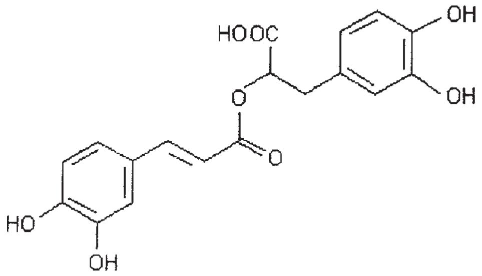

Figure 1 Rosmarinic acid.

procedure (Peterson and Alfermann, 1988). Quantities of phenolics indicating rosmarinic acid have been extracted from various *Salvia* plant cells such as *S. officinalis* and *S. fruticosa*, with different concentrations of the phenylalanine precursor in the medium (Panagiotopoulos *et al.* manuscript in preparation). The approximate quantitative determination of RA has been done by measuring the absorption at 333 nm, the characteristic wavelength of phenolic rings of the callus ethanolic extract. Another report (Hippolyte, 1990, see also Chapter 16 in this volume) gave an alteration of production by 50% with different hormonal balances in the medium for *S. officinalis* suspension culture.

Lithospermic acid B (LSA)

Studies about lithospermic acid B (Fig. 2) as a *Salvia* plant extract have been made mainly in roots and rhizomes of *S. miltiorrbiza* Bunge. One of them (Fung *et al.*, 1993) achieved isolation to > 95% purity by HPLC from the aqueous extract of the roots of the plant. This study involved a demonstration of the myocardial salvage effect of LSA. Another report gave a comparative reference of seventeen different *Salvia* species and varieties that gave a wide range of amounts of LSA, from 0.3 µtg/mg of extract at *S. deserta* to 258.3 µg/mg at *S. paramiltiorbiza f. purpureo ruba,* measured by LC-MS analysis (Kasimu *et al.*, 1998).

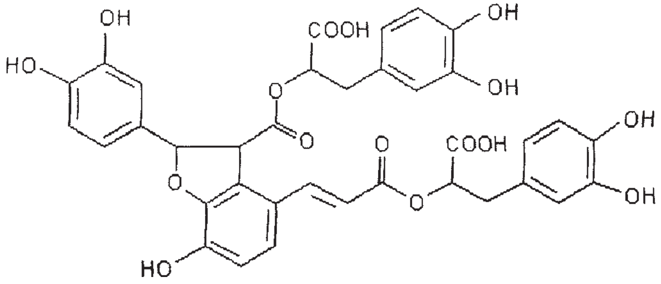

Figure 2 Lithospermic acid B.

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Salvianolic acids

Salvianolic acids A (Sai A), B and K are some of the different chemical structures in *Salvia* plants (Figs 3, 4, 5), all belonging to the phenolics group. Observed for the first

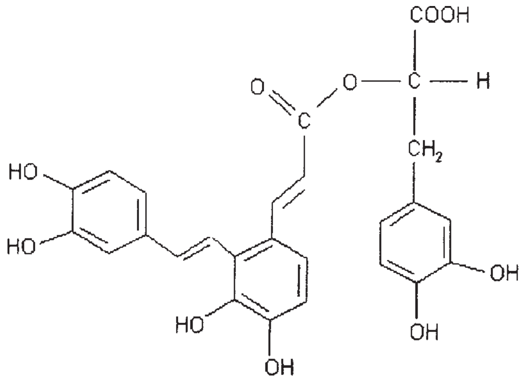

Figure 3 Salvianolic acid A.

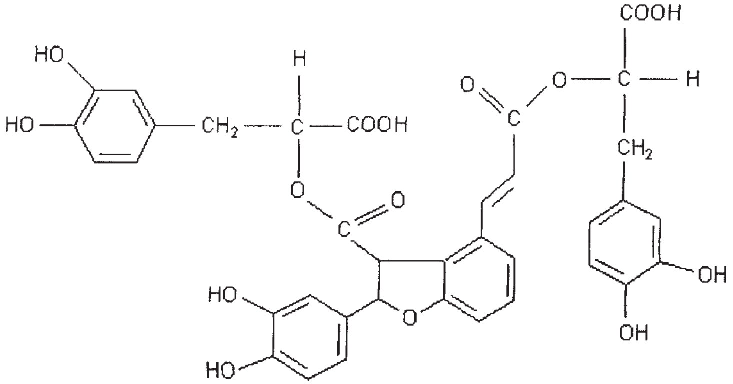

Figure 4 Salvianolic acid B.

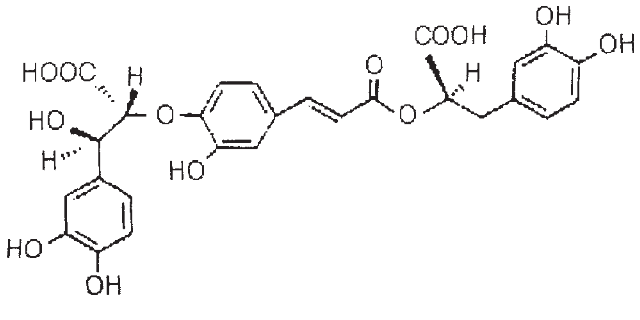

Figure 5 Salvianolic acid K.

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time in roots of *S. miltiorrhiza* B. (Li *et al.,* 1984), Sai A was investigated for its protective action against peroxidative damage to biomembranes (Liu *et al.,* 1992). Some interesting work has been made, involving anti-oxygen radicals activities in rats (Lin and Liu, 1991), effects of Sai A on oxygen radicals released by rat neutropilis and on neutrophilic action (Lin *et al.,* 1996) and generally antioxidant activities of salvianolic acid. Quantitative determination gave considerable amounts (29.28 µg/mg) of salvianolic acid K in *S. deserta,* compared to other species (Kasimu *et al.,* 1998).

Concerning the terpenoids group, there have been reported many of them in *Salvia* plants. Particularly worth-mentioning is a report on the isolation of twenty one abietatriene diterpenes grouped in five classes and found in only two *Salvia* species : *S. canariensis* and *S. mellifera* (Moujir *et al.,* 1993). Those compounds were evaluated for structure-antimicrobial activity. Most of known *Salvia* terpenoids have been evaluated for anti-fungal, antiviral and generally antimicrobial activity. We are going to refer to the most common terpenoids which are:

Tanshinones

An extensive report on tanshinones, (Hu and Alfermann, 1993) in hairy root cultures of *S. miltiorrhiza* B., gave an quantification of tanshinone I (Fig. 6), tanshinone II_A (Fig. 7), tanshinone II_B (Fig. 8), tanshinone V (Fig. 9), tanshinone VI (Fig. 10), cryptotanshinone (Fig. 11) and dihydrotanshinone I (Fig. 12), by means of a quantitative HPLC. The cultures were established by sterile grown plants whose roots were transformed by infection of five strains (only four gave roots) of *Agrobacterium rhizogenes*. Production of cryptotanshinone was achieved by a series

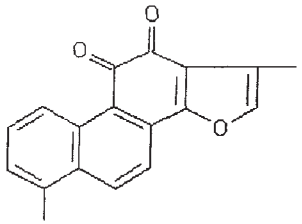

Figure 6 Tanshinone I.

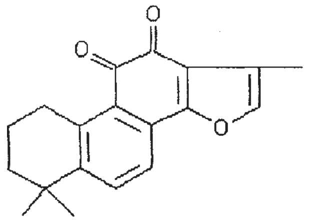

Figure 7 Tanshinone II_A.

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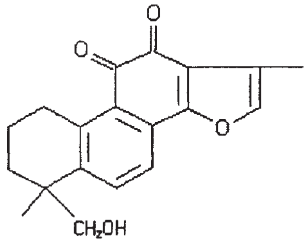

Figure 8 Tanshinone II_B.

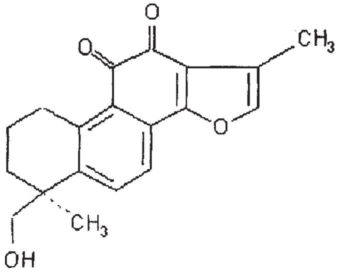

Figure 9 Tanshinone V.

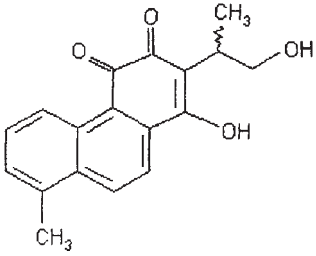

Figure 10 Tanshinone VI.

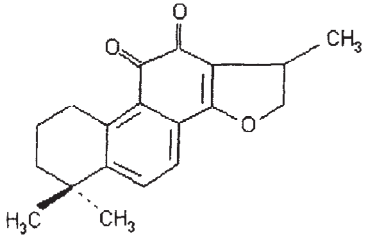

Figure 11 Cryptotanshinone.

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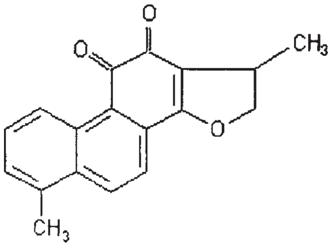

Figure 12 15, 16-dihydroxytanshinone I.

of suspension cultures with callus from seedlings of *S. miltiorrhiza* B., comparing an original MS medium without Fe-EDTA and a simplified medium that finally gave better production of cryptotanshinone (110±4.86 mg/l) (Miyasaka *et al.,* 1989).

Ferruginol

In the previous reference (Miyasaka *et al.,* 1989), the production of ferruginol (Fig. 13) was also achieved but with a better production in the original medium (69.3±.77 mg/l). Hu and Alfermann (1993), also recorded the production of ferruginol from transformed root cultures of *Salvia miltiorrhiza* B., by leaf explants without hormones in the medium, reaching a maximum of approx. 3.4 mg/g of dry weight. The most productive strain of the *Agrobacterium rhizogenes* was used for inducing hairy roots from the leaf explants.

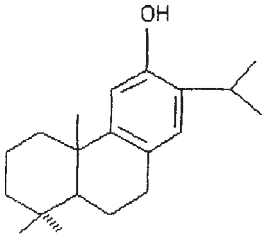

Figure 13 Ferruginol.

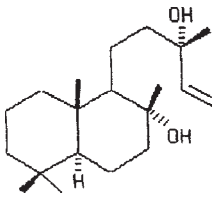

Figure 14 Sclareol.

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Sclareol

Another diterpene, sclareol (lab-14-en-8, 13(S)diol) (Fig. 14) is used commercially in the manufacture of ambergris perfumes and is also a potent inhibitor of growth of rust fungi (Baily *et al.,* 1975). Accumulation of sclareol by cell cultures of *S. sclarea* has been achieved at rates (μg/g per day) varying from 0.2 to 6% of those found in the parents plants. The maximum accumulation was shown to take place near the entry to the exponential growth phase (Banthorpe *et al.,* 1990).

Carnosic (carnosolic) acid and carnosol

It has been reported that carnosol (Fig. 15) and carnosic acid (Fig. 16), among many other antioxidants, can give about 90% of the antioxidant activity of rosemary plants (*Rosmarinus officinalis* L.) (Aruoma *et al.,* 1992). Studies showed that carnosic acid gave the strongest inhibitory effect, among other substances extracted from *R. officinalis* L. (including carnosol), on HIV-1 protease in cell-free assays (Paris *et al.,* 1993). Skin tumorigenesis was inhibited by carnosol, again from rosemary plants (Huang *et al.,* 1994). In *Salvia* species, those two diterpenes where isolated from seven-day-old *in vitro* grown plantlets of *S. canariensis,* though neither carnosol nor carnosic acid could be obtained from 25-day-old plantlets (Luis *et al.,* 1992).

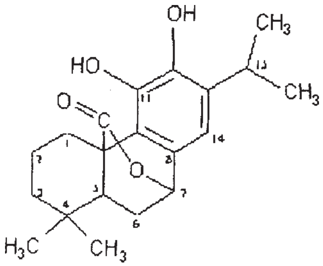

Figure 15 Carnosol.

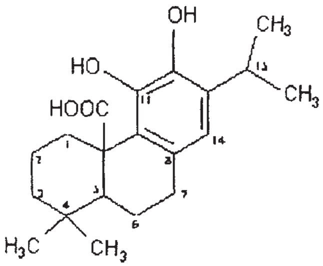

Figure 16 Carnosic acid.

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Underlying that many other terpenoids (as well as phenolics) have been also isolated from *Salvia* plants and studied for various biological activities, we point out the interest in producing those medicinally important substances on industrial scale.

SECONDARY METABOLITE PRODUCTION USING LIQUID CULTURE

Liquid Culture and the Mechanisms of Plant Secondary Metabolism

Suspension cultures have been frequently used for the production of secondary metabolites from various plants. The usual procedure is to transfer a callus from a solid medium to a liquid one (*i.e.* in conical flasks), establishing a suspension culture. Many works have been made to determine the parameters for the optimisation of secondary metabolite production, varying medium composition, culture conditions and using different plant species and varieties.

In each plant two conditions have to be met before a considerable production of secondary metabolites can be occur: the various enzymes directly involved in secondary metabolism have to be inducted and also sufficient supply of precursors from primary metabolism is necessary before product accumulation can be observed. Primary metabolism not only supplies secondary metabolism, but many of the same precursors are important to the synthesis of cell constituents. For that reason there is often a competition for these precursors between the growth process and secondary metabolism. As a consequence, synthesis of secondary metabolites occurs especially when growth is slow or absent, often after the completion of a differentiation process (van der Plas *et al.,* 1995). It is often observed that secondary production is induced when plant cells are under stress, a reaction that refers to the physiological role of secondary metabolites. Liquid culture has the advantage of easier control of culture conditions so that with continuous suspension cultures we can obtain specific growth stage treatments to enhance the production of the secondary metabolite of interest.

Enzymatic mechanisms were examined in rosmarinic acid (RA) formation in *Anchusa officinalis* cell suspension cultures (Mizukami and Ellis, 1991). As expected, the RA concentration increased progressively during the late linear growth phase, reaching a maximum in the stationary phase. The same metabolite (RA) in *Salvia officinalis* and *S. fruticosa* gave similar results concerning the phase that the maximum production was achieved (Panagiotopoulos *et al.* manuscript in preparation). In the same work the use of phenylalanine precursor, as well as higher sucrose quantities in the liquid medium gave a slight support to the production of RA. Suspension cultures were also used by Hu and Alfermann (1993) for the production of diterpenoids (tanshinones and ferruginol) from *Agrobacterium rhizogenes*—trans formed leaf segments of *Salvia miltiorrhiza*. In this study a considerable percentage of the secondary metabolites was found in the liquid medium. In another report of Miyasaka *et al.* (1989) involving *in vitro* production of cryptotanshinone and ferruginol, secondary metabolites passed from the plant cells to the medium in a remarkable percentage, especially when immobilized cells were cultured (see below).

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Cell Immobilization in Liquid Culture

There has been much interest in the use of immobilized cultured plat cells for biotransformation and production of secondary metabolites. According to Miyasaka *et al.* (1986), the production of the diterpenes cryptotanshinone and ferruginol by immobilized cultured cells of *Salvia miltiorrhiza* was successful. The two diterpenes were produced continuously by immobilized cells using a two-stage culture method, with normal medium for growth and then medium without Fe-EDTA to suppress cell growth an induce the production of the two metabolites. For immobilization, cells were entrapped on calcium alginate beads (mean diameter of 4mm) and after 25 days their productivity was calculated. In comparison with the free cell culture, production of cryptotanshinone and ferruginol by immobilized cells was about 39 and 61% respectively, of the yield obtained from the free cells. Nevertheless, about 74% of cryptotanshinone was released into the medium, whereas only 25% was released by free cells. A similar "release effect" (the mechanism of this effect is unknown) was also reported for alginate entrapped cultured cells of *Catharanthus roseus* (Brodelius *et al.,* 1979). After the re-use of the immobilized cells, they retained their viability but the total production of the diterpenes was lower, proba bly due to accumulated lipophilic metabolites. This considerable attribute of some particular secondary substances can give a different perspective to the production of individual metabolites with continuous cell suspensions, by simplifying the isolation process of the desirable secondary product, without the need of reestablishment of the culture after extracting the product.

CONCLUSION—PERSPECTIVES FOR SCALE-UP PRODUCTION

The commercial production of natural products from cell or tissue culture has long been a goal for plant biotechnology. Though a great deal of effort has been applied, only a handful of process has come to commercial fruition, principally in Japan, and even in those instances production units operate at the limits of economic viability. In spite of apparent lack of progress at the commercial level it is generally agreed that the potential of the synthesis of high value natural products from plant cell cultures is tremendous (Stafford and Fowler, 1991).

Bioreactors are in-wrought with the large scale production of secondary metabolites, so it is obvious that study and optimisation of liquid culture can lead to the desirable result. Stirred tank and airlift bioreactors are the most common in plant cell cultivation for secondary metabolites production. An example of bioreactor configuration developed by Wilson *et al.* (1990) is seen at Figure 17. At this point we can mention a 200 1 bioreactor configured as a module spiral stirrer that produces rosmarinic acid from cultivated cells of *Coleus blumei*. Large scale processes based upon plant cell cultures have been reported even up to 75m^3 of a stirred tank bioreactor, growing cells of *Rauwolfia serpentina* (Westphal, 1990). Two-stage process formats, including cell immobilization, seem to be the most possible and effective way to produce extracellular secondary metabolites from *Salvia* plants. Already evaluated bioreactor configurations can be tested in *Salvia* plants, giving to individual secondary substances the potential for an industrial scale production.

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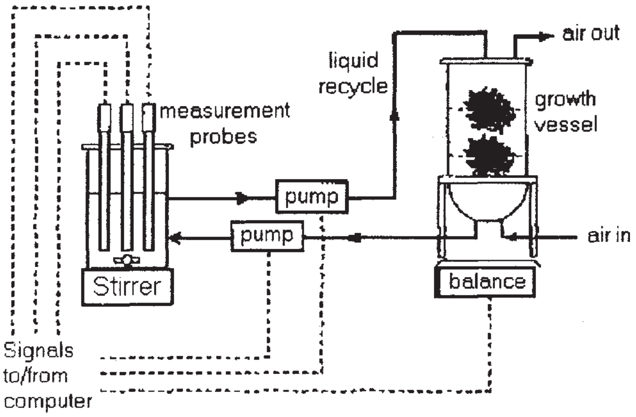

Figure 17 Droplet reactor as developed by Wilson *et al.* (1990) for transformed root cultures.

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VII. COMMERCIAL ASPECTS
19. PRODUCTION OF *SALVIA* OIL IN MEDITERRANEAN COUNTRIES

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INTRODUCTION

Salvia L. (Labiatae) is an important aromatic genus which is frequently used as herbal tea and as a source of essential oils and aromachemicals.

Salvia species of commerce include *Salvia officinalis* L., *S. fruticosa* Miller (Syn. S. triloba L. fil.), *S. lavandulaefolia* Vahl., *S. verbenaca* L. and *S. sclarea* L. *S. tomentosa* Miller is another species with development potential. While *Salvia officinalis* is cultivated worldwide, and *S. sclarea* in Europe and North America, *S. fruticosa* oil is produced from wild plants.

THE SITUATION IN MEDITERRANEAN COUNTRIES

Spain

In Spain, *S. lavandulaefolia* (Spanish sage) oil is produced from cultivated plants transplanted from the wild in Murcia, Albacete, Granada and Almeria by cottage-type stills in 0.8–1.0% yield. In recent years, 40 tons (1993, 25 tons (1994), 30 tons (1995) and 40 tons (1996) of Spanish sage oil have been produced (Mirales, 1998).

Morocco

In Morocco, leaves of *S. officinalis* and *S. verbenaca* are exported and no sage oil is produced. Dried leaf exports from Morocco, in recent years, are as follows: 124.5 tons (1993), 139 tons (1994), 82.4 tons (1995) and 81.4 tons (1996).

Israel

In Israel, there is no commercial scale production of sage oil.

Egypt

In Egypt, while leaves of the cultivated *S. officinalis* are incorporated in herbal teas, there is no production of sage oil.

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Portugal

In Portugal, there is no sage oil production. Wild crafted *S. argentea, S. sclareoides* and particularly *S. verbenaca* var. *algarvica* are used in the preparation of herbal medicines.

Italy

In Italy, *S. officinalis* and *S. sclarea* are cultivated, but there is no sage oil production at commercial scale.

Yugoslavia

In old Yugoslavia, sage oil used to be produced from cultivated *Salvia officinalis*. No information has been obtained about the current situation.

France

In France, *S. sclarea* cultivation was started in the Nimes district. Later, the cultivation was expanded to the Alpes-Maritimes, Bases-Alpes and in the Department of Var. Today, the most important area of cultivation is still the Department of Var. Clary sage oil was distilled by Roure Bertrand et Fils (now, Givaudan-Roure, Inc.) and France was a leading producer of the oil by the late 1950s. In 1994, France produced only 4 tons of clary sage oil (Lawrence, 1994).

Greece

In Greece, there is no commercial scale distillation of sage oil although *S. fruticosa* is a native plant.

Turkey

Turkey is an exporter of sage *(Salvia fruticosa)* leaves. Sage is collected from the wild in western part of Turkey, dried and exported. There is also a sizeable internal market for sage leaves, since they are used as herbal tea in western and southern provinces of Turkey. Sage leaf exports of Turkey (kg/US$/unit value in dollars) are as follows:

In Turkey, sage oil is produced from *S. fruticosa* (wild) in Manisa and Alanya by steam distillation using commercial stills. The oil also known as Elma yagl (Apple oil) is obtained using cottage-type stills in South-Aegean parts of Turkey. In some districts, the plant is locally known as "elma" (apple) due to the resemblance of galls

1991	*1992*	*1993*	*1994*	*1995*	*1996*
508.646	563.863	576.257	400.220	547.821	681.044
1,096.145	1,118.746	1,367.658	837.511	1,121.261	1,476.674
2.160	1.980	2.370	2.090	2.050	2.170

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growing on its leaves and stems to small apples. Another gall forming sage is *Salvia pomifera,* which is not used in Turkey either as herbal tea or for essential oil production. Its essential oil contains thujones as main constituents (Baser, 1993). Sage oil is locally used medicinally as a herbal oil. Annual production is an estimated 500 kg (Baser, 1994).

Table 1 Distillation conditions of the plant materials.

Material	*Pilot Plant Charge (kg)*	*Process Time (h)*	*Steam Rate (kg steam/kg mat.–h)*	*Total Oil (ml)*	*Pilot Plant Yield (%)*	*Lab. Yield (%)*
S. triloba	198	4	0.6	4274	2.2	2.3
S. triloba	198	4	0.6	4476	2.3	2.3
S. triloba	197	4	0.6	4594	2.3	2.3
S. sclarea	32	3 1/2	0.8	143	0.4	0.5
S. sclarea	24	3 1/2	0.8	91	0.4	0.5
S. sclarea	30	3 1/2	0.8	104	0.4	0.5

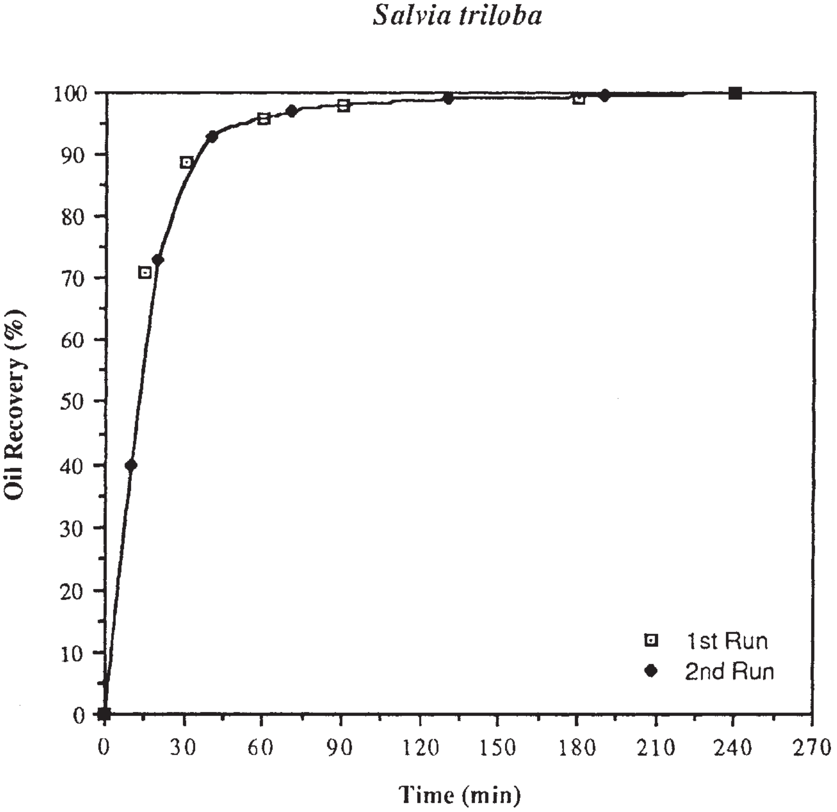

Figure 1A

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PILOT PLANT SCALE PRODUCTION

Pilot plant scale and semi-commercial scale production trials of *S. fruticosa* (dried leaves) and *S. sclarea* (flowering tops) oils were carried out at TBAM using 500 L and 2000 L capacity stainless steel steam distillation plants. Processing conditions are given in Table 1. Oils were collected in time intervals and analyzed by gas chromatography/mass spectrometry (GC/MS) using fused-silica capillary columns.

Salvia fruticosa

In *S. fruticosa,* oil recovery reached a maximum after 60 minutes, and only 10% more oil was obtained between the 60th (1 h) and 240th (4 h) minutes (Fig. 1a). An interesting pattern was observed in the appearance of the three main constituents. In the first 15 minutes, 1, 8-cineole content reached a peak (63%) and gradually decreased to 8% after 4 h. Camphor content reached its maximum (16%) in 30th minute, while ß-caryophyllene content started increasing from 4% (15 min) to 24% (90 min), and decreased to 17% after 4 h (Fig. 1b).

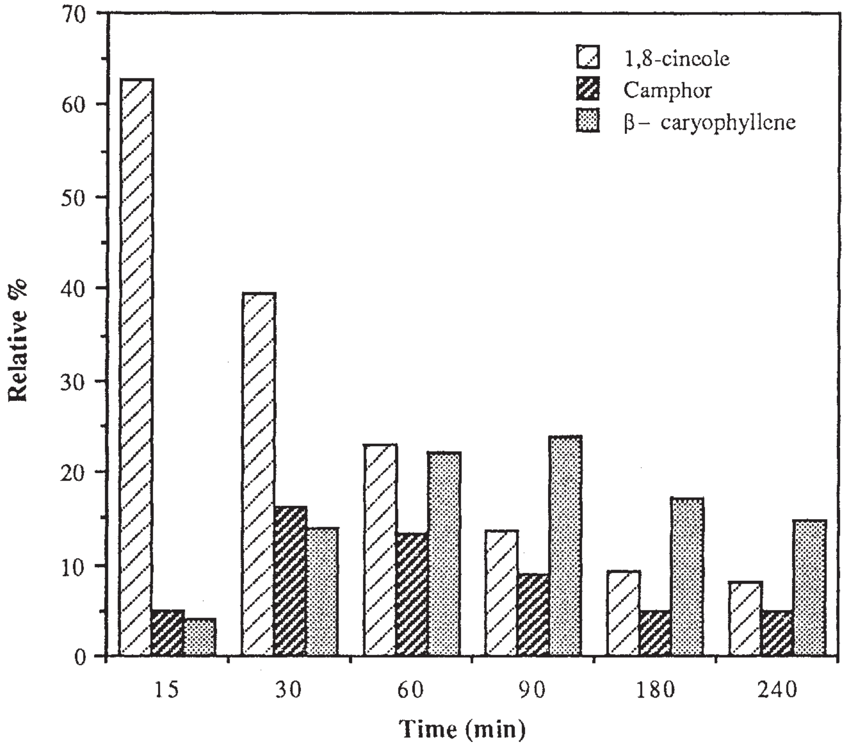

Figure 1B

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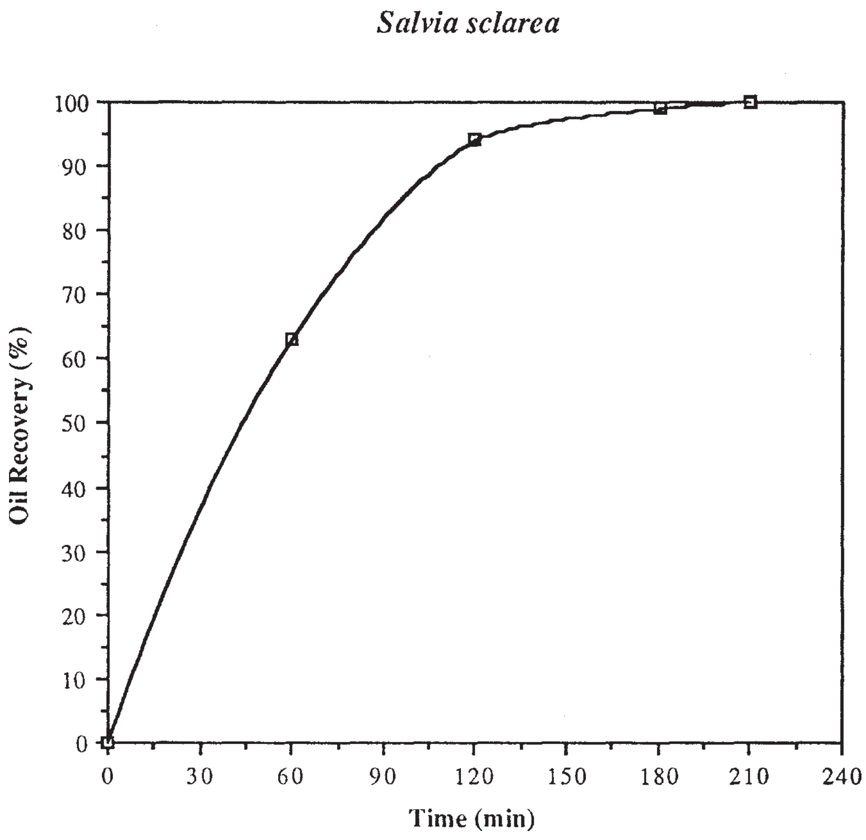

Figure 2A

Salvia sclarea

Although *S. sclarea* is a native plant, its oil is not produced at commercial scale in Turkey. Pilot plant scale trials at TBAM provided information on its process parameters. In the first 2 h, 90% of the maximum oil yield was recovered within the total distillation time of 4 h (Fig. 2a).

Maximum linalyl acetate content of 72% was observed in the first hour of distillation, and varied between 67–72% throughout the distillation period. Terpinyl acetate content reached a maximum (10%) after 3 h. Linalool content, on the other hand, gradually decreased from 6% to 3.5% at the end of the distillation period (Fig. 2b).

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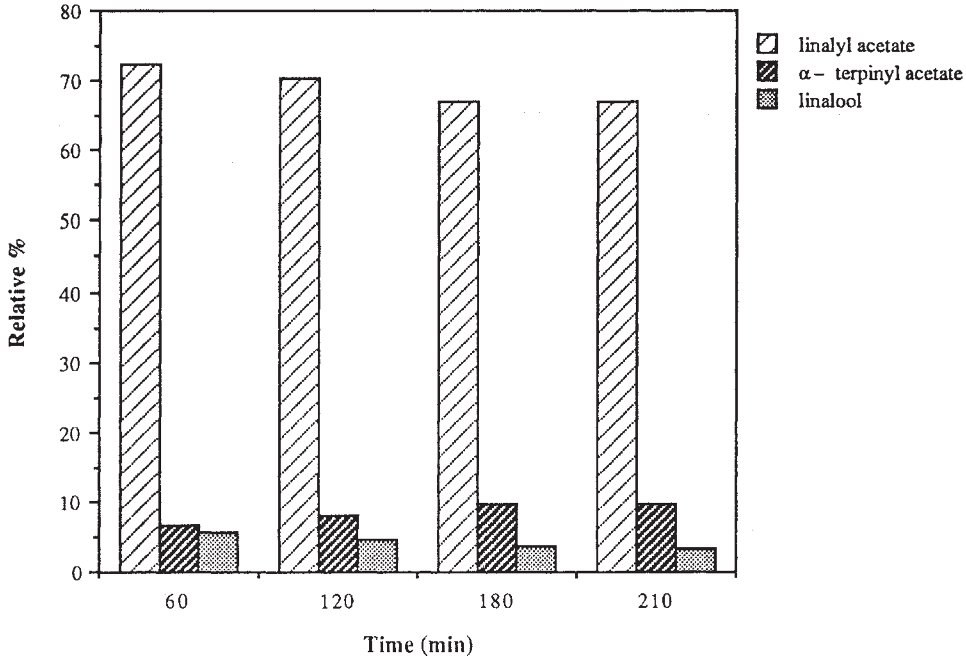

Figure 2B

ACKNOWLEDGMENTS

The author is grateful to Drs. Mine Kurkcuoglu, S.H.Beis and T.Ozek of TBAM for their help in pilot plant scale trials and analysis of the oils.

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VIII. GENERAL ISSUES
20. SCIENTOMETRIC ANALYSIS OF SCIENCE AND TECHNOLOGY BIBLIOGRAPHIC INFORMATION SOURCES WITH REGARD TO GENUS *SALVIA*

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INTRODUCTION

Researchers have been, for the past decades, facing an ever accelerating growth of all kinds of scientific and technical information. With the advent of electronic technologies and especially the Internet the data flow has turned into a deluge. This is affecting especially interdisciplinary fields. It has gradually become impossible to even follow the distribution of relevant information let alone to study such infor mation. Information is scattered across many "meta" sources. Moreover, the same information is being stored differently in different sources so it cannot be retrieved by using common search strategy. As storage systems and meta sources grow so do the strategies.

End-users are increasingly at a loss as to which sources to turn to in order to retrieve as much relevant information as possible. Kuhlthau (1997) admits that, while all users sustain some degree of anxiety, such anxiety or sense of frustration is even much higher with those who are involved with interdisciplinary sciences. The field of medicinal plants is in its characteristics a typical interdisciplinary or transdisciplinary (crossdisciplinary) field within the life sciences. It is composed of agricultural, biomedical, biological, chemical and other aspects. Many times it implies also cultural and social dimensions so end-users must expect such information to be scattered across variety of disciplines and pertaining information sources. We cannot in detail present all the sources where such information might appear. We will introduce only some major life-sciences databases where information is presented in bibliographic way, that is on the level of title, informative abstract, keywords and publication data. Bibliographic databases still represent by far the most important source of science and technology information. Data in such databases are organized in a systematic manner so it is possible to study well the scatter of information. To some extent bibliographic data on medicinal plants were studied by Gupta (1990) who investigated subject-based publication activity indicators for medicinal and aromatic plants established from Indian Medicinal and Aromatic Plants Abstracts. Zhang (1994) studied distribution of Chinese traditional medicine references in Medline database. In our study we will identify most major bibliographic life-sciences databases and assess them as pertinent sources for information on the genus *Salvia*. On the example of *Salvia* we will observe the degree

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of overlap across databases. We will also identify the annual trend of publishing for this genus and major journals where *Salvia*-related articles had been published. We will also investigate these databases and their differences in relation to keyword or classification representation of the topics.

INFORMATION RETRIEVAL, PRECISION AND RECALL

Users frequently believe that they possess sufficient skills to independently browse different bibliographic databases. These are indeed structured in a rather similar way. However, when it comes to controlled indexing language the users are often unaware of many traps they might be exposed to. This is particularly the case with transdisciplinary fields, where thorough users wish to browse or search as many relevant sources as possible and do not wish to overlook any of them. Will experiences that a user acquires with one database be of significant help with another? This may be the case if the user is a very regular and competent patron of the databases in question. If the user is only an occasional customer of such a service this may do him or her more damage then good because such a user can acquire a false impression as to the real potential of a database. We are now of course talking about the end-user's independent utilization of an information service, especially if the user has not become skilled in the use of search tools provided by the service. The trap lies especially in the databases being made increasingly and indiscriminately available via WWW.

When a user faces a large database of several million references he or she invariably wonders what is the number of relevant references/documents that could potentially be retrieved from such a database. The user will then wish to perform such a search as to maximize the number of those relevant references. With a very high number of retrieved references, however, many references will turn out to be incorrect and will be labelled as noise. These were not sought by the user and can be very costly because with most online services it is necessary to pay each retrieved reference.

Success and noise of a search in relation to prior assignment of subject headings were scientometrically first pursued by Cleverdon (1962). The measures of retrieval success are generally summarized as recall and precision. Recall is the proportion of relevant items that were retrieved in relation to all relevant items, i.e. also those that were not retrieved in a search. Precision is the proportion of relevant items that were retrieved in relation to all the items that were retrieved. The above concepts have been much discussed in scientometrics. Absolute relevance is namely difficult to assess and different documents will be judged as relevant by different users (Van Rijsbergen, 1981). To increase recall some authors (Gomez, 1990) suggest several techniques. Such techniques can in many cases greatly enhance precision. However, if the user wants to maximize recall he runs the risk of significantly reducing the precision. Problems of relevant and nonrelevant items retrieved in relation to information seeking behaviour by end-users were studied in detail by Saracevic (1988).

In order to maximize recall as well as precision of information retrieval, i.e. to maximize the number of relevant documents and minimize the noise, it is of utmost importance that users acquire good prior knowledge as to how the databases are compiled, structured and indexed. In our further text we will seek to present some

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particularities of each of the above databases with relation to compilation, and indexing of *Salvia*-related documents. Hereby we wish to stress the importance of familiarity with the process of data extraction and the importance of services being offered by information professionals in libraries or information centres.

Let us first point to one of the major bibliometric issues, namely the indexing consistency which additionally affects precision and recall of information retrieval. Indexing, which is as a process closely related to classification, is usually defined as assigning, usually by information professionals—indexers, of controlled terms such as descriptors (keywords) or subject categories to documents. Cognitive problems in indexing have been addressed time and again so we will present only some illustra tive conclusions. Even the same indexer, let alone two or more, may assign quite different descriptors to the same article in a different period of time. Some finds report that two different indexers on average assign as few as 50% of identical descriptors to the same documents. (Sievert and Andrews, 1991). With regard to the high subjectivity of indexing Quinn (1994) even presents a view of extreme subjectivists who hold that indexing is an unresolvable problem in information science. Besides subjectivity, Lopes (1996) summarizes some more internal and external barriers that affect indexing: terminological aspects that express the com plexity and dynamics of the natural language information; options of controlled language which are limited within the system; concept of the subject that depends on conceptual foundations of a subject catalogue. Hurt (1997), however, calls for renewal and further development of indexing systems and classification. We cannot here go into details as to indexing of same aspects in the same article across several databases. We will, however, present a few examples which will illustrate vast differences that exist amongst databases in assigning of terms to a specific topic.

Success of searching inevitably becomes precarious as soon as end-users desire to formulate complex queries that involve Boolean OR as well as AND and when searches involve an object and several aspects of the studied object. With our study on genus *Salvia* we carefully selected the most appropriate search technique in order to maximize the recall. With filtering techniques we consequently also managed to optimize the precision.

METHODS: DATABASE SELECTION, DATA EXTRACTION, COMPILATION AND ANALYSIS OF THE GENUS *SALVIA*

Number of databases grows almost monthly so we are presenting only major information systems/databases in life sciences. These databases generally hold several million references each and register annual growth of up to several hundred thousand. They have been in service for many years and have gained recognition world wide. With numerical data we provide some approximation as to the state in the 1998, though information on number of references, annual growth and coverage period may vary in different database directories. We present data that are generally valid for CD-ROM versions. Online versions sometimes cover longer periods and more data. With most databases there exist also older printed versions for early periods. Moreover, some of the services may compile some additional databases with

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data on selected specific documents such as conference proceedings or reviews. As we cannot in detail expand all such possibilities we provide WWW URLs of all the database compilers so the users can get prompt and constantly updated information on coverage and access possibilities to different products.

Most references in these databases are of bibliographic type and scientific and technical in nature. With increased WWW accessibility it is to be expected that a growing number of full text papers will be linked to bibliographic references. However, there are serious copy-right issues to be considered, with authors as well as publishers involved, so even though there exist technical possibilities these will not so soon lead to absolute global online accessibility of abstracted material that is being compiled in these databases. This will remain even more so the case with older documents because very high expenses are involved with inputting of analog data.

In the preceding and following text we sometimes interchangeably use terms documents, references and records. Records are usually considered as database references that were derived from a particular document. A term citation is sometimes also used in this context. The later, however, is more appropriate with respect to "cited" references, i.e. "quotations" which were not subject of our research.

We have not extended our review into the field of legal and market-related issues and pertaining specialized databases. Some of the databases, listed beneath, do provide exhaustive information on documents such as patents or standards. End-users should nevertheless consider also other sources to acquire further information of this type. Most larger academic and special libraries will possess enough expertise to offer additional instructions to this end.

Presentation 1:* *The list of databases understudy

AGRICOLA covers mostly US agricultural and life sciences information and is compiled by National Agricultural Library, USA. (records: 3.500.000, annual growth: 100.000, span: 1970-present) *http://www.nalusda.gov*

AGRIS covers world-wide agricultural and life sciences information and is compiled by some 140 national and international centres in coordination by FAO (records: 2.300.000, annual growth: 130.000, span: 1975-present) *http://www.fao.org/waicent/waicente.htm*

BIOLOGICAL ABSTRACTS (BIOSIS) covers world-wide life sciences (biology, medicine) information and is compiled by BIOSIS in USA and UK (records: 3.500.000, annual growth: 350.000, span: 1985-present. There is an additional database on reports, reviews and meetings) *http://www.biosis.org*

CHEMICAL ABSTRACTS covers world-wide chemical and biochemical information and is compiled by Chemical Abstracts Service in USA (records: 14.000.000, annual growth: 650.000, span: 1967-present) *http://www.cas.org/*

CAB ABSTRACTS covers world-wide agricultural and life sciences information and is compiled by CAB International in UK (records: 3.300.000, annual growth: 160.000, span: 1973-present) *http://www.cabi.org/*

DERWENT BIOTECHNOLOGY ABSTRACTS world-wide biochemical, pharmacological and related information (many patents) and is compiled

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internationally by DERWENT (records: 220.000, annual growth: 16.000, span: 1982-present) *http://www.derwent.com/*

EMBASE (Excerpta Medica) covers world-wide biomedical and pharmacological information and is compiled by Elsevier Science in the Netherlands (records: 3.700.000, annual growth: 350.00, span: 1980-present) *http://www.elsevier.com/*

FSTA (Food Science and Technology Abstracts) covers world-wide information on food science, food technology and human nutrition and is compiled by IFIS (International Food Information Service) which is sponsored internationally by four organizations (CABI, DLG, IFT, PUDOC) (records: 510.000, annual growth: 20.000, span: 1969-present) *http://www.ifts.co.uk/*

ISTP (Index to Scientific and Technical Proceedings) covers information on worldwide life sciences and technical conference proceedings. It is compiled by Institute for Scientific Information (ISI), USA (records: 800.000, annual growth: 160.000, span: 1991-present), *http://www.isinet.com/*

LIFE SCIENCES COLLECTION covers world-wide information on life sciences is compiled by privately owned CSA (Cambridge Scientific Abstracts), USA (records: 1.500.000, annual growth: 130.000, span: 1982-present) *http://www.csa.com/*

MEDLINE (MEDlars onLINE) covers world-wide biomedical information and is compiled by National Library of Medicine, USA (records: 8.500.000, annual growth: 300.000, span: 1966-present) *http://www.nlm.nih.gov/nlmhome. html*

NAPRALERT—(NAtural PRoducts ALERT) is a relational database and covers world-wide literature on the chemical constituents and pharmacology of plant, microbial and animal (primarily marine) extracts. It is compiled by the Program for Collaborative Research in the Pharmaceutical Sciences, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy of the University of Illinois at Chicago, (online only, records: relational data, 116.000 documents, annual growth 7.000, span: 80%—1975-present, 20%—1650–1975) *http://pcog8.pmmp.uic.edu/mcp/Welcome.html*

SCISEARCH (Science Citation Index) is a multidisciplinary citation database and covers world-wide life sciences and technical information. It is compiled by Institute for Scientific Information (ISI), USA (records: 13.800.000, annual growth: 700.000, span: 1980-present). ISI compiles also other major databases such as Current Contents, *http://www.isinet.com/*

The above databases are available on CD-ROMs, via online services and also via WWW. Electronic publishing (e.g. CD-ROMs) and commercial distribution facilities for many of the above databases are provided by SilverPlatter *http://www.silver platter.com/*. Those databases all use the same software (SPIRS). ISI databases use ISI software. Most databases are largely accessible also via several online information providers such as

DATASTAR *http://www.krinfo.ch/krinfo/products/datastar/index.htm;*

DIALOG *http://www.krinfo.ch/krinfo/products/dialog/index.htm*

DIMDI (Deutsches Institut fuer Medizinische Dokumentation und Information) *http://www.dimdi.de/*

STN International (the Scientific and Technical Information Network) *http://www.cas.org/stn.html*

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These services offer comprehensive online catalogs for all types of databases they assist and can be consulted also on patents, standards, legislation, etc.

In order to estimate characteristics of the above databases we carried out a complex scientometric analysis of all possible references to the genus *Salvia*. Except for the Chemical Abstracts and Biological Abstracts we chose with all other databases a ten-year period (1986–1995) what can illustrate the chosen object well enough. Our finds are multifold so we will be able to present only some selected results. These will, however, offer a comprehensive insight into the area of *Salvia-related* documents and citations. With most databases we used CD-ROM versions. With Embase, Chemical Abstracts, and Napralert we searched via online or WWW providers. The searches in the Chemical Abstracts were so costly that we could not afford to pay the estimated ten-year costs to perform an experimental search only. With an unrefined search there were namely more than 1.000 hits in this large database. So we resigned ourselves to choosing a 1991–1992 period. As Biological abstracts on CD-ROM were available for the 1991 and onwards period, and an online search was costly again, we considered the limited 1991–1995 period only. For ISTP we could also access 1991–1995 period only but the overall number of references was very low with this database. However, for easier parallel comparison we selected 1991–1992 period for both Biological and Chemical Abstracts in most charts. With the generous help of the (Napralert) provider, we were able to obtain cost-free data from this database via the Internet.

We compiled a common experimental database that enabled us to investigate relations amongst references synthesized from particular documents, which can appear as records in one or several databases simultaneously. To set up our experimental database we used MS-Access. We further analyzed the records with MS-Excel and MS-Statistica.

RESULTS

Usage of Key-Words/Descriptors and Subject Categories

Before compilation of our experimental database we examined thoroughly the existing indexing systems of the databases. Most databases employ elaborate indexing language. In most of the cases it consists of keywords as well as broader subject categories. There are usually only a few dozen subject categories in a database. Keywords are in most cases derived from thesauri which are controlled glossaries of several thousand (even tens of thousands) discipline-related terms that provide for consistent indexing. Some of the databases, though, don’t employ a thesaurus and make use of non-controlled free terms only. In some databases there exist both controlled terms (descriptors derived from a systematic thesaurus) as well as free terms (identifiers assigned freely).

Keywords in some databases can occupy as many as four fields. With CAB there are controlled descriptors, organism descriptors, and broader terms, as well as noncontrolled identifiers. This system was introduced in the early nineties. Before that the descriptor field was only one. SCI introduced descriptors as well as identifiers only in the nineties. Agris provides “explode” possibilities. All broader terms from the hierarchical tree are automatically allocated to each indexer-assigned “narrow”

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descriptor from the thesaurus. Also, the Latin term is meant to be assigned as an index term to the plant before harvest, i.e. during the vegetation period, and the English term is valid for the plant or plant product (tuber, seed, fruit…) after it has been harvested, and has usually been processed or placed in a store room. Such distinction is logical with most field crops, and a search on Solanum tuberosum/-diseases as opposed to potato/diseases yields quite different results. This distinction is, however, somehow blurred with medicinal plants: A usage of freshly cut *Salvia* and usage of dried "sage" is not so well defined. It is nevertheless possible that the scientific term *"Salvia"* appears nowhere in the record. This is quite habitually the case with the FSTA database where the end-users should be aware of less frequent usage of scientific terms and should always apply also the English term "sage" for *Salvia*. Medline has been employing its highly structured MeSH thesaurus for many years. This indexing system offers complex "explode" utility where a single term can be significantly expanded by a hierarchical tree-structure, so many narrower terms can be automatically included in the search. *Salvia* or sage, however, don't feature as descriptor terms in this database. Documents can therefore be retrieved solely with the help of the free-text terms, i.e. free-text of titles and abstracts. In all other data bases *"Salvia"* as well as "sage" can be used as an indexing and search term. How ever, this usage is not interchangeable. The majority of documents, except in the FSTA database, is indexed with the scientific term *Salvia* only. Still, there are with all databases quite a few documents where the scientific term *Salvia* never appears in the record, so these references can be retrieved only by the search term sage.

It is important to turn some attention also to the usage of the broader term for the entire group of medicinal or spice plants. There exists a variety of terms in the above databases. (Agris) uses almost exclusively the "drug plants" term. Other databases use "medicinal plants". There is also a number of other terms that may or may not co-appear with the term for a specific genus or species. The most frequent terms are herbs, essential oil plants (crops), condiments, flavourings, culinary herbs, flavouring crops, spices and also herbal remedies, herbal medicine, traditional medicine, her baceous drugs etc.

With our analysis we sought to evaluate the *Salvia-related* record as thoroughly as possible. We performed many test searches in each of the databases to prepare optimal search strategy and to maximize recall (percentage of all existing relevant documents). We therefore formulated our search request for both SALVIA and SAGE bound together with the Boolean OR. We searched in all the content fields, such as title, abstract and keywords (descriptors, identifiers). Especially with sage we expected lower precision turnout, i.e. more "noise". However, as we sought to establish maximum recall, we decided to acquire all the references, and then to refine our searches "manually" by deleting the noise. As expected there was much sage-related noise. These were mostly documents on *sage grouse (birds),* and *sage scrub* or *sage brush*. In a few cases *sage* stood for wise. S.A.G.E. appeared many times also as an acronym, particularly in the Chemical Abstracts. Especially in medical databases there was also quite some noise with *Salvia*. Namely it was *saliva* that was misspelled as *Salvia*. It comes logically to one's mind that saliva is much more "in use" in the medical databases than *Salvia* so hence the mistyping on the part of information specialists.

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In our experimental database we compiled from the above databases more than 3.500 *Salvia* or sage related documents for the ten-year period. This number of course included all the duplicates (many multiple duplicates). This database served as our main apparatus. When all the duplicates were filtered we were left with some 1.600 distinct references which we then compiled in another database. To analyze Biological and Chemical Abstracts in relation to other databases we compiled an additional 1991–1992 database.

Key-Word Distributions for the Same Documents Indexed by Different Databases

In our database we could sort documents in many different ways. One way was to sort them according to the frequency of appearance of the same document in different databases. Thus we identified all duplicates and especially multiple duplicates. This duplicated references/records enabled us to identify indexing characteristics in each of the databases. In the Presentation 2 we display the titles of three selected documents together with a selection of the most significant keywords and category codes for each database to illustrate consequential differences that exist among databases for indexing of the same document.

In the previous chapter we mentioned that the keyword fields in the databases differ in number of fields and subfields as well as in structure. To portray the indexing structure we keep original delimiters for each of the databases to offer an additional insight as to the structure of these fields. As there may be with some databases (esp. Biological Abstracts and Chemical Abstracts) many terms in the keyword fields, we present, in order to provide some common denominator, only those terms that most clearly represent the main topic of the article. We chose, if available though, all the terms for the species, genus or group (medicinal plants, drug plants, etc.). The field names are presented as in the original databases (DE-descriptors, ID or IDEN-identifiers, ENDE-English descriptors, IT-indexing terms, OT-organism descriptors, BT-Broader terms…). All these names represent the concept of the keywords in the particular database. ID usually stands for non-controlled free terms that are not derived from a thesaurus and have been assigned *ad hoc*. CC always stands for the category codes or the subject categories. These are broad subject representations of the main concept in the document in question. These broader concept categories are not available with all the databases.

Presentation 2:* *Examples of same documents indexed in different databases

Article Title: BIOSYNTHESIS OF MONOTERPENES—INHIBITION OF (+)-PINENE AND (-)- PINENE CYCLASES BY THIA AND AZA ANALOGS OF THE 4R-ALPHA-TERPINYL AND 4S-ALPHA-TERPINYL CARBOCATION

SCI
ID: sage, *Salvia*-officinalis; adenosyl-1-methionine…enzymatic cyclization; obtusifoliol isomerase; gas-chromatography

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MEDLINE
DE: Aza Compounds/cs [Chemical Synthesis], Aza Compounds/pd [Pharmacology], Indicators and Reagents, *Isomerases/ai [Antagonists & Inhibitors], [Isolation & Purification], Kinetics, Molecular Structure, Organophosphorus Compounds, Terpenes/cs [Chemical Synthesis, Metabolism], *Plants/en [Enzymology], [Pharmacology]

EMBASE
CT: biosynthesis*; plant*; enzyme inhibition*; enantiomer*; plant leaf; stereochemistry; enzyme kinetics; terpene*; synthetase*; IT: plant; nonhuman...

CHEMICAL ABSTRACTS
ST: pinene cyclase inhibition terpinyl carbocation analog; aza analog terpinyl carbocation pinene cyclase; IT Kinetics, enzymic... RL: BIOL (Biological study) (inhibition of (+)- and (-) RL: SPN, Geranyl pyrophosphate RL: (Synthetic preparation);... (Reactant) (redn. of, by (+)- and (-)-pinene cyclases, mechanism of)...

CC: 7–4 (Enzymes) Section cross-reference(s):

AGRICOLA
DE: *Salvia*-officinalis, monoterpenes-, biosynthesis-, enzymes-, enzyme-activity, stereochemistry-, kinetics-, inhibition-

CC: plant physiology and biochemistry

AGRIS

ENI: *Salvia*-officinalis, *monoterpenes-; *biosynthesis-;*enzymes *chemistry-; *inhibition-ENC: biochemical-reactions; chemical-reactions; chemicophysical-properties; labiatae-; monoterpenoids-;

CC: Plant-physiology-and-biochemistry

BIOLOGICAL ABSTRACTS

DE: *Salvia* officinalis; 4r-alpha terpinyl; geranyl pyrophosphate; (dextro)-alpha-pinene; inhibition kinetics; enantioselectivity BC: Labiatae ST: Plants...

CC: Enzymes-Chemical-and-Physical; Metabolism-General-Metabolism-Metabolic-Pathways, Plant-Physiology-, Biochemical-Studies-General

Article Title: GROWTH SUPPRESSION AND RAISED TISSUE CHLORIDE CONTENTS IN AMMONIUM-FED MARIGOLD, PETUNIA AND *SALVIA*.

BIOLOGICAL ABSTRACTS

DE: *Salvia* splendens; plant; crop industry; horticulture; ammonium toxicity; nitrogen nutrition BC: Labiatae; ST: Plants;

CC: Nutrition-Malnutrition-, Plant-Physiology-Biochemistry-and-Biophysics-, Horticulture-Flowers-and-Ornamentals, Phytopathology-Nonparasitic-Diseases...

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CHEMICAL ABSTRACTS
ST: *Salvia;* chloride plant ammonium toxicity, Mineral wool, Soil substitutes, Plant stress (ammonium excess, growth suppression…), RL: BIOL (Biological study) (absorption of..), IT: Nitrate, Potassium;
CC: (Fertilizers, Soils, and Plant Nutrition)

SCI
DE: *Salvia-Spkndens;* (NH4)+-(NO3)-Ratio; (NH4)+ Toxicity ID: nitrate; nutrition; nitrogen; plants

AGRICOLA
DE: *Salvia-,* phytotoxicity-, stress-, suppression-, growth-, ion-activity, nutrient-solutions, ammonium-, chlorides-, nitrates-.

AGRIS
ENI: **Salvia-;* *phytotoxicity-; *stress-; *growth-; *ions-; *nutrient-solutions; *ammonia-; *chlorides-; *nitrates- ENC: biological-development; compositae-; inorganic-compounds; labiatae-; salts-; solanaceae-; toxicity- IDEN: *Salvia*-splendens
CC: Miscellaneous-plant-disorders; Fertilizing

CAB
DE: nutrition-; nitrogen-; toxicity-; Soilless-culture; nutrient-solutions; composition-; ornamental-herbaceous-plants OD: Ageratum-houstonianum; ID: *Salvia-splendens* BT: ornamental-plants;
CC: Plant-Nutrition; Plant-Production

Article Title: A NOVEL ANTIMICROBIAL ABIETANE-TYPE DITERPENE FROM SALVIA ALBOCAERULEA

CHEMICAL ABSTRACTS
ST: *Salvia* albocaerulea IT: Antibiotics Fungicides and Fungistats, Diterpenes and Diterpenoids RL: (Biological study), isolation and structure and antibiotic activity of IT: Sage, S. albo-caerulea RL: (Biological activity or effector, except adverse) antimicrobial activity of)
CC: 11–1 (Plant Biochemistry)

EMBASE
CT: phytochemistry*; antibacterial activity*; plant ; drug isolation; drug structure; diterpene*/drug comparison/drug analysis/drug development; unclassified drug
IT: plant; nonhuman, therapy

MEDLINE
DE: Animal, Anti-Infective Agents/ch [Chemistry], [Isolation & Purification], [Pharmacology], [Drug Effects], Diterpenes/ch [Chemistry],
plants, medicinal [Chemistry],

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AGRICOLA
DE: saliva- (saliva: SIC), plant-extracts, pharmaceutical-products, diterpenes-, antimicrobial-properties, structure-activity-relationships.

CC: plant physiology and biochemistry, agricultural products(plant)

AGRIS
ENI: **Salvia*-; *drug-plants; *diterpenoids-; *antimicrobial-properties; *chemical-composition; *chemical-structure; *leaves- ENC: chemistry-; crops-; labiatae-; plant-anatomy; IDEN: *Salvia*-albocaerulea;

CC: Plant-physiology-and-biochemistry

SCI
id: constituents; medicine

Frequency Distributions of References in Different Databases

In order to facilitate graphic and further textual presentation we will use two-letter acronyms for the databases under study:

ac	Agricola	em	Embase
as	Agris	fs	FSTA
ba	Biological Abstracts	is	ISTP
ca	Chemical Abstracts	Is	Life Sciences Collection
cb	CAB Abstracts	me	Medline
db	Derwent Biotechnology Abst.	np	Napralert
sc	Scisearch		

We present some of the results in two time series. The first series was used for the entire period of 1986–1995 which does not include Biological and Chemical Abstracts, and ISTP. The second series uses 1991–1992 period and includes all databases. In Figures 1 and 2 we present distribution of *Salvia*-related documents as references in each of the above databases.

In Figures 3 and 4 we present distribution of documents/references covered in selected groups of databases. We clustered Agris, Agricola, CAB and FSTA into the agricultural group (agr). We then clustered Embase and Medline into the medical group (med). For comparison we again included specialized database Napralert, and multidisciplinary database SCI (1986–1995), and Biological and Chemical Abstracts (1991–1991). Within each cumulative bar we also present number of documents that appear exclusively in the selected database or database group and thus cannot be found outside the group or the database. These illustrate the level of non-overlap in databases.

We see that the highest number of references is to be found in agricultural databases Agicola, Agris, CAB and FSTA. These databases also contain the highest number of references that are not to be found in any of the databases outside this

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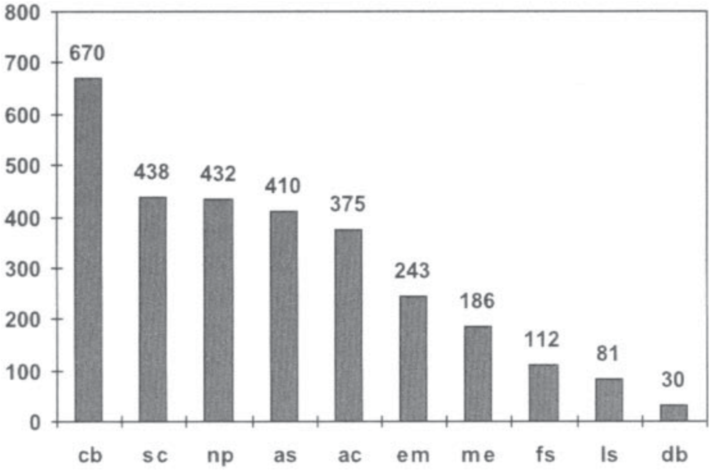

Figure 1 *Salvia-related* references in selected databases in the period 1986–1995.

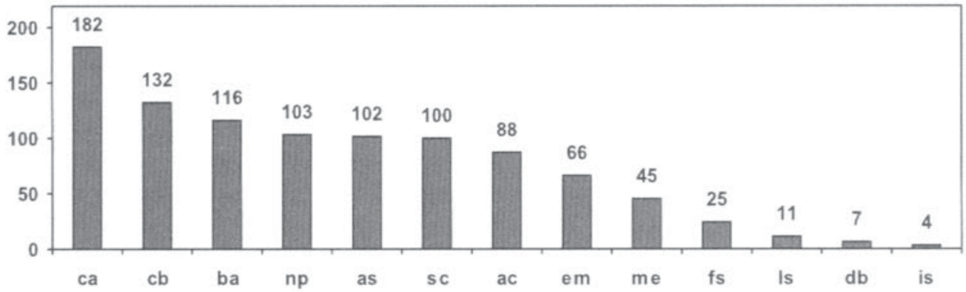

Figure 2 *Salvia*-reiated references in all databases in the period 1991–1992.

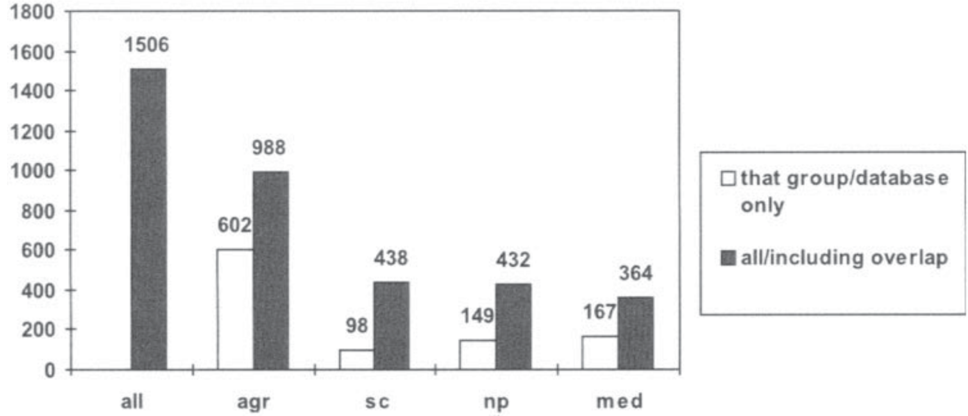

Figure 3 Overall and single appearance of references in databases and selected groups of databases in the period 1986–1995.

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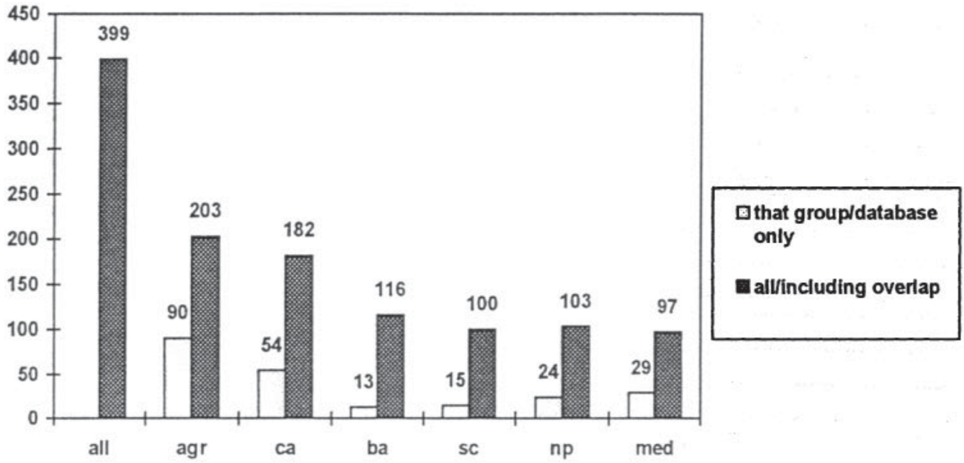

Figure 4 Overall and single appearance of references in databases and selected groups of databases in the period 1991–1991.

group. Chemical Abstracts also display very high coverage, however, as will be seen in Table 4 almost 20% of *Salvia*-related references in this database were obtained from patents.

In Tables 1 and 2 we present co-appearance of references in the databases and groups of databases.

Table 1 Co-appearance of references in databases and selected groups of databases in the period 1986–1995.

	agr	*med*	*np*	*sc*
agr	988	142	229	292
med	142	364	124	112
np	229	124	432	197
sc	292	112	197	438

Table 2 Co-appearance of references in databases and selected groups of databases in the period 1991–1992

	ba	*agr*	*ca*	*med*	*np*	*sc*
agr	116	74	76	44	48	63
bt	74	203	84	43	50	66
ca	76	84	182	49	67	68
med	44	43	49	97	36	32
np	48	50	67	36	103	46
sc	63	66	68	32	46	100

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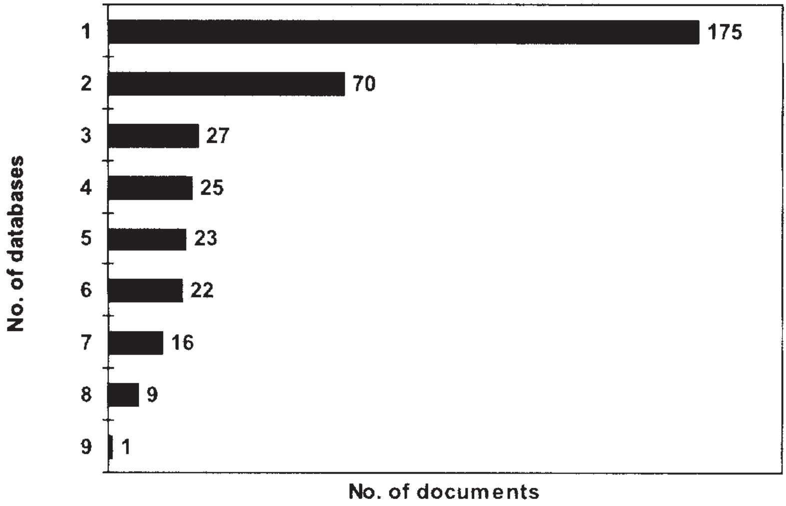

Figure 5 Overlap for a single document in 13 databases in the period 1991–1992.

In Figure 5 we present bars that illustrate the extent of the overlap amongst the investigated 13 databases in the period 1992/1992. Bars represent the number of single documents, duplicates or multiple duplicates. It is possible to find *Salvia*-related references in all of the above databases, however, we see that the maximum overlap is only nine databases, and this is represented by only one reference. That means that only one document is represented as a bibliographic record in this many databases. Even though there are many multiple duplicates a majority of documents (175) appear as single records in only one database what can also be seen as the lower bars in the Figure 4. In order to better compare database overlap for similar documents we, with the data in the Figure 5, excluded patents, standards and theses, which are specifie kinds of documents and are not to same extent represented in all databases. We can generally conclude that the overlap-level is quite small. There is also no single database that could be regarded as the most important one, and which would cover the majority of all *Salvia*-related documents, esp. if we consider some older experiments (Martyn and Slater, 1964) where 85% of all relevant records were expected to be covered by single most effective abstracting service. Tenopir (1982), however, expects to find 60–70% of the relevant literature in one database but he concedes that a multidisciplinary subject may involve higher number of databases.

Our experimental database that filtered all the record overlap presented us with remarkable opportunity to estimate the real annual rate of *Salvia*-associated publishing. This was namely established from commutative data based on as many as ten major databases. Furthermore, we were able to investigate whether there exists some trend based on the ten-year observation period. The trend turned out to be even more uniform than we ever expected (Table 3, Figure 6). Regression analysis (95% confidence limits) yielded a growth of 5, 7 references per year. Average yearly growth was 2.2%. For regression analysis we excluded year 1995 which is probably

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Table 3 Yearly distribution of the *Salvia*-related non-duplicated references for the period 1986–1995.

Year	*No. of ref.*
1986	144
1987	117
1988	142
1989	139
1990	139
1991	149
1992	185
1993	163
1994	172
1995	156

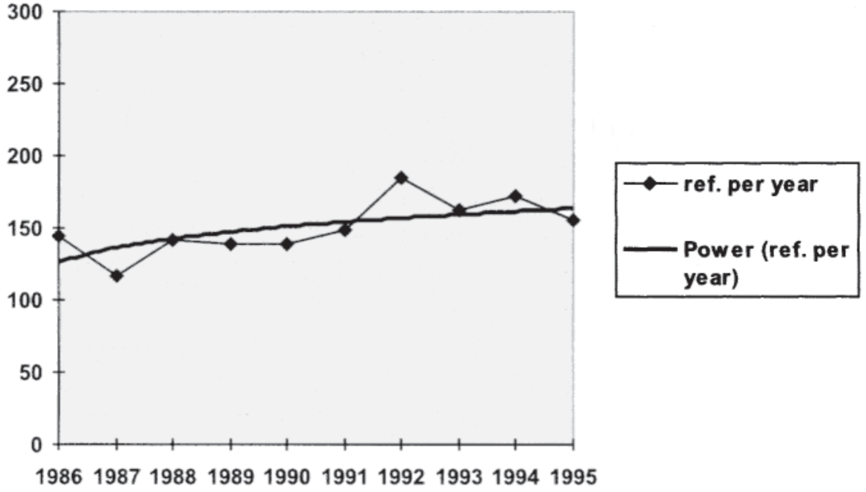

Figure 6 Yearly growth of the *Salvia*-related non-duplicated references for the period 1986–1995.

not yet complete and would slightly distort the data. The trend line that we identified was an important part of our study as it very well reveals slight but constant growth of research in the area.

Distribution of References in Primary Sources

Growth trend was estimated on the basis of all references regardless of the document type. However, we also wished to present distribution of references per document type (Table 4, Figure 7). In our experimental database we assigned each reference/record a uniform type that we contrived on the basis of bibliographic information

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Table 4 Distribution of references by document types in the databases ac, as, cb, db, em, fs, Is, me, np, sc (1986–1995), ca (1991–1995), ba, is (1991–1995), all (1991–1992).

	ac	*as*	*ba*	*ca*	*cb*	*db*	*em*	*fs*	*is*	*ls*	*me*	*np*	*sc*	*all*
aj	362	374	115	144	604	21	242	95		79	185	419	431	330
ap	10	21	1	6	49	1	1	2	7	2	1	6	7	23
m	1	11		2	14			2				2		9
s		4						6						1
p				26	2	8		7				56		30
t	2			4	1									6

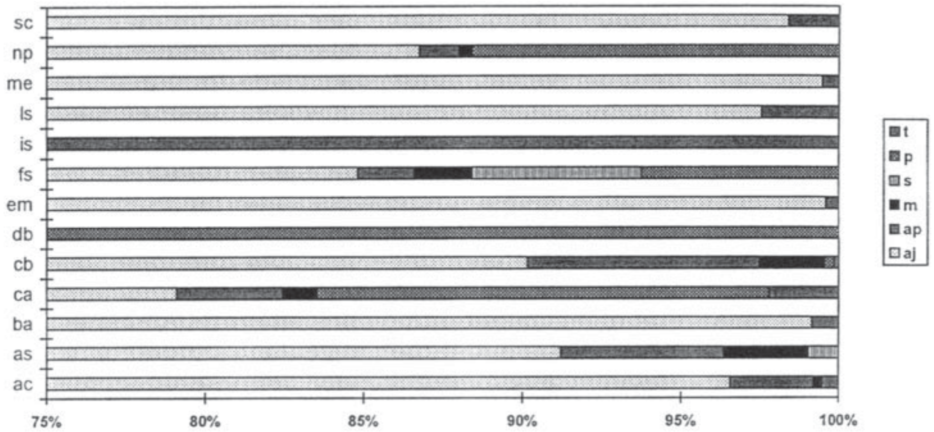

Figure 7 Distribution of references by document types in the databases ac, as, cb, db, em, fs, Is, me, np, sc (1986–1995), ca (1991–1995), ba, is (1991–1995) as percentage of all references in each database.

derived from records. We used common chart for all databases, however, again the data for Chemical and Biological abstracts and the ISTP were applicable for limited periods only (1991–1992 and 1991–1995 respectively). We cumulatively calculated the record type for 1991–1992 only. (For sumarized data for each database compare also Table 1 and Table 2)

We considered the following document types (dt): article/journal—aj, article/-proceedings—ap, monograph—m, patent—p, standard—s, thesis—t.

From the Table 4 and Figure 7 it is evident that overwhelming majority of references is derived from journal articles. Some databases, however, offer many references from other primary sources. Napralert, end especially Chemical Abstracts (the later based on two-year observation) contain many references to patents. Also, Chemical abstracts hold as many as four theses where *Salvia* is mentioned. Agris and FSTA are good sources of references to standards.

Journals therefore represent by far the most important source of *Salvia*-related data. We thus wished to identify core journals which yielded the majority of articles.

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Table 5 List of journals with five or more *Salvia*-related references in the period 1986–1991.

Journal	*Articles*
Phytochemistry	116
Planta Medica	47
Chinese Journal of Modern Developments In Traditional Medicine	37
Acta Horticulturae	31
Journal of Natural Products	28
Fitoterapia	26
Archives of Biochemistry and Biophysics	23
Hortscience	21
Journal of Ethnopharmacology	21
Flavour Fragrance Journal	18
Journal of Essential Oil Research	17
Tetrahedron	17
Phytotherapy Research	14
Acta Pharmaceutica Sinica.	13
Chemical and Pharmaceutical Bulletin/	12
Journal of Organic Chemistry	11
Journal of Biological Science Research	10
Journal of Agricultural and Food Chemistry	9
Perfumer and Flavorist	9
Taxon	8
International Journal of Pharmacognosy	8
Journal of Traditional Chinese Medicine	8
China Journal of Chinese Materia Medica	7
Chinese Medical Journal	7
Korean Journal of Pharmacognosy	7
Journal of the American Oil Chemists' Society	6
Pharmaceutica Acta Helvetiae	6
Pharmazie	6
American Journal of Chinese Medicine	6
Chinese Medical Sciences Journal	6
Chinese Pharmaceutical Journal	6
Trudy Vsesoyuznogo Nauchno Issledovatel'skogo Instituta Efiromaslichnykh Kul'tur	6
Econ Bot	5
Journal of the American Society for Horticultural Science	5
Journal of Plant Physiology	5
Rastitel'nye Resursy	5
ACS-Symposium-Series	5
Herba Hungarica	5
Journal of Environmental Horticulture	5
Journal of the Pharmaceutical Society of Japan	5
Plant Disease	5
Sciences des Aliments	5

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In Table 5 we present the list of journals by five or more *Salvia*-related articles per journal in the period 1986–1995. Again this list is only an approximation as it was construed on the basis of references from the ten databases of the 1986–1991 period.

Given the overlap ratio, and good representation of *Salvia*-related records in the ten major databases, we believe that the number of references per journal based on all databases might slightly change but the journal-list would probably not with an exception of addition of a few titles.

DISCUSSION AND CONCLUSIONS

In our research we observed fairly even distribution of *Salvia*-related bibliographic references across several databases. We can therefore conclude there exists no single superior database which could account for majority of relevant references. However, to some extent we may assume that the highest number of *Salvia*-related references is to be found in the agricultural databases if considered as a group but even here a mere 50% of all relevant documents can found. The degree of overlap amongst databases is fairly low and roughly half of all references are to be found in one database only. The presence of these references in such a number of different life-sciences-related electronic information sources demonstrates high degree of inter-disciplinarity of the subject and can serve as a reminder to end-users as to which information sources not to overlook. As expected by far the highest number of references was derived from journal articles. There exist, however, some differences as to the type of article amongst databases. ISTP database is based entirely on proceedings and consists solely of congress articles.

With the above data we must nevertheless take into account that the citations were obtained by searches that were based on free text. Thus the databases furnished with more informative free-text data such as abstracts rendered respectively higher number of references. In order to maximize recall we were not able to apply any other search strategy. Finally, we were interested in the overall (cumulative) number of all *Salvia*-related references. Our free-text strategy across all the databases yielded more than three thousand documents, which after having been filtered, enabled us to assess the growth trend in the field. Growth is slight but persistent and shows a continuing presence of interest in the field.

The majority of the sources/databases examined in our study are probably accessible at any large university. End-users can thus quickly find a way of obtaining well-organised data and eventually acquire entire documents. Most of the databases are available on CD-ROMs as well as via specialized online providers. They are also available for individual online/WWW searches. Here, though, we need to extend some additional warning. In case of online searches the end-user will be usually billed per one hit what can quickly amount to a significant sum especially if the retrieved documents did not meet user's expectations. As we could see the databases are structured quite differently and will for an identical document offer different search possibilities. The same search strategy will, on the other hand, offer different search results in different databases. The indexing language in some of the databases is

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namely so complex that it can to full extent be used only by very skilled information professionals in that field. Such professionals are familiar with many characteristics of a well-structured indexing language that can, however, mislead a poorly skilled end-user.

In our article we sought to provide some insight into the occurrence of *Salvia*-related references in different databases. We hope the users will find some of the above information useful with their research. We gave some special emphasis to many traps that may dwell in complex data storage so users should know what (not) to expect and get some prior knowledge as to the structure of each of the electronic sources. This is becoming even more acute in the era of the Internet when information is becoming much more readily available. Traditional bibliographic databases are structured in a very sophisticated way and yet each of those employs different search devices. With the Internet we may expect the data to diffuse into many more directions, and in a much more disorderly way. The end-users may believe that with new technologies they can circumvent information services, however, in the end we strongly invite the users to co-operate with such services which will also be able to point to many other relevant information sources that could otherwise escape attention of end-users. Information professionals will be glad to help with extensive experiences they gathered in the process of information retrieval and may in their way significantly contribute to success of a research.

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APPENDIX

SHORT NOTES ON THE OLFACTORY PROPERTIES OF THE ESSENTIAL OIL OF SOME SAGE SPECIES

1. *Salvia officinalis* (Dalmatian sage)

Odor and flavor: fresh-herbaceous (due to thujones), warm-spicy and penetrating, somewhat camphoraceous (due to camphor), with a pleasant, sweet-herbaceous dry-out.

Application: in mixtures with lavandin, rosemary, citrus oils and bois de rose oil. As an additive in aldehydic perfume bases and spicy fragrances.

2. *Salvia sclarea* (Clary sage)

Odor: strong and pungent, usually persisting on skin, clothes and perfume bottles but disappearing during steam-distillation. The oil, which is highly volatile, has been described to resemble the odor of cistus oil, Moroccan chamomile, tobacco or tea.

Application: in perfumery and in aromatherapy.

3. *Salvia lavandulaefolia* (Spanish sage)

Odor: fresh-herbaceous, resembling the odor of cineole, camphor, eucalyptol or pine.

Application: in soap perfumery, in detergent fragrances and as a freshener in industrial perfumes, as well as in mixtures with the oil of rosemary, lavender, spike lavender, pine needle and citronella.

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